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No. 1. — *Observations on Budding in Paludicella and some other Bryozoa.* By C. B. Davenport.[1]

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A. SPECIAL PART.

I. Introduction.

The somewhat heterogeneous studies here brought together have been prosecuted at different times and in different places, as opportunity for getting light on the problem of non-sexual reproduction as exhibited in the group of Bryozoa has presented itself.

While studies on the fresh water species were pursued chiefly here at Cambridge, those on marine Bryozoa were made while occupying one of the tables of the Museum at the United States Fish Commission Labora-

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy, under the direction of E. L. Mark, No. XXVIII.

tory at Wood's Holl, Mass., during the summer of 1889, and while at Mr Agassiz's Newport Laboratory during the summer of 1890. To my instructor, Dr. E. L. Mark, for many valuable suggestions during the progress of my work and the writing of this paper, to Mr. Alexander Agassiz, for the kind hospitality accorded me at his Newport Laboratory, and to Hon. Marshall McDonald, United States Commissioner of Fish and Fisheries, and Dr. H. V. Wilson, Assistant at Wood's Holl, for favors shown me while at the Wood's Holl Laboratory, I make grateful acknowledgment of my indebtedness.

A word as to localities. The marine Bryozoa were found especially abundant at Newport on floating eel-grass in the cove and on the piles of the wharf. The embryos of Cristatella and Plumatella were found in colonies which literally covered the bottom of some parts of the south or shady side of Trinity Lake, Pound Ridge, New York. They occur especially in densely shaded and fairly deep water near the shore.

The Gymnolæmata present many difficulties to finer technique. They possess a chitinous covering, often very thick, and frequently, in addition, a calcarous skeleton. When the latter is present, picro-nitric acid mixed with sea water is a fairly good fixing reagent; when it is absent, hot corrosive sublimate was most serviceable. The objects must be transferred through the grades of alcohol with extreme caution, to prevent the collapse of the ectocyst. I used the chloroform-paraffin method of embedding in order to make transfers more gradual at this stage. Some difficulty was experienced in staining such small objects on the slide, since the tissues are very loosely associated; and on the other hand *in toto* staining is unsatisfactory in some cases, owing to impenetrability of the ectocyst. Often it was necessary to open the body cavity of each individual by means of a sharp knife or needle. The best results were obtained with alcoholic dyes like Kleinenberg's hæmatoxylin and Mayer's cochineal; although Ehrlich's hæmatoxylin was often used with success.

II. Budding in Paludicella.

1. Architecture of the Stock.

Paludicella, as is well known, occurs in quiet streams and forms stocks on the under surfaces of stones and other objects. Seen with the naked eye these stocks appear as a fine lacework, composed of constantly branching lines of individuals. Some of the stocks which I have measured are over 25 mm. in length along their greatest diameter.

When the stock is studied more carefully, it is seen that the individuals which compose it are arranged one in front of the other, forming lines. (Figs. 1, 2, 2ª.) We may distinguish (1) a single primary branch, which forms a continuous line from the oldest individual, which has been derived directly from the egg, to the terminal one; and (2) secondary branches, which arise from the individuals of the primary branch and at right angles to their axes. Typically, a secondary branch arises from both the right and left sides of each adult member of the primary branch, but in some cases the secondary branch of only one side appears to be formed. The secondary branches are composed, like the primary, of a continuous line of individuals placed end to end. These in turn give rise to tertiary branches, which run out at right angles to the right and to the left of the secondary ones, and hence parallel to the primary branches. Quaternary branches may occur in like manner, but I have never seen branches of a higher order than the fourth. All of these branches may lie in one plane, but frequently some of the lateral buds are so placed that they give origin to secondary branches which rise above the plane of the object upon which the stock lies. A study of Figure 1 and the corresponding diagram, Figure 2, reveals some additional facts. The two lateral buds of an individual do not arise at the same time, and there is a tendency for the first, and therefore oldest and most developed, secondary branches to arise alternately on opposite sides of the primary branch. This last rule has many exceptions, however.

The long axis of the individual coincides with that of its branch; the sagittal plane lies in that axis, and at right angles to the substratum. The atrial opening is near the distal end of the individual in the sagittal plane, and is turned away from the substratum. The anal aspect of the polypide is placed nearer the tip of the branch, — hence distad; the mouth, on the contrary, proximad.

A very casual observation shows that not all branches nor all individuals are of the same size. The shortest and therefore youngest branches are placed most distally, and are seen as small buds. The terminal individuals of the branches are also evidently less well developed than the more proximal ones. The adult individuals measure from 1.5 to 2.0 mm. in length and from 0.30 to 0.35 mm. in width. The younger individuals differ from the older in form also. The outline of the adult branch, looked at from the side, and disregarding the atrial opening, is formed by a series of beautiful sigmoid curves (Fig. 9). The concave and convex points of the upper and lower sides of an individual are not placed exactly opposite each other, and the lower (abatrial) side approximates more

nearly to a straight line. The point at which the upper and lower curves most nearly approach each other is where the separation of two individuals takes place; that at which they are farthest apart is the middle of the zoœcium, occupied by the polypide and sexual organs. The outlines of the young zoœcia are straighter, and their breadth is considerably less than that of the adult.

From what we have already seen, the method of growth of the stock is perfectly evident: it is by the formation of new median buds at the tips of existing branches, and of new branches from lateral buds. In order to understand the origin of the individuals of the primary branches, to which subject we will first turn our attention, we must study the tips of the branches.

2. Histology of the Budding Region.

Figures 7–9 will serve to show more in detail the method of formation of new terminal individuals. We find in these cases one polypide already pretty well developed and attached to the body wall by means of the kamptoderm at about the point at which the pyramidal muscles (*mu. pyr.*) are seen to be forming. That portion of the animal which extends from about the region of formation of the muscles to a point a little proximad of the tip represents the region which will go to form the new individual. The tip itself, for reasons which will presently appear, is not to be included in the terminal individual. The tip of the branch is to be regarded as homologous with the margin of the corm in corm-building genera of Gymnolæmata. Figures 7–9 (*gn.*) also show the position of the bud which is to produce the polypide. By consulting first Figure 9, in which the polypide bud is apparent, the significance of the swellings of the body wall in Figures 8 and 7 becomes clear.

Figure 14 (Plate II.) represents a stage in the development of the polypide bud, somewhat later than that shown in Figure 9, and this may serve us as a starting point in our study of the origin of a new individual, and, first of all, of the new polypide. The whole of Figure 14, from the tip down to the neck of the older polypide (*cev. pyd.*), may be divided, for convenience, into three zones: first, that distad of the young bud, which may be called the *tip of the branch* (Fig. 14, α to β); secondly, the region of the bud itself, which may be called the *gemmiparous zone* (β to γ); and thirdly, the region between this last zone and the neck of the older polypide, which, for want of a better name, may be called the *proximal zone* (γ to δ). In the formation of a new polypide between a and β, that region will in turn become divisible into the three zones just named,

exactly as the region *a* to *δ* represented the *tip* of the branch when the older polypide, whose neck is shown at *cev. pyd.*, was of the age that the younger bud is now. It will be necessary first of all to study carefully each of these three regions before treating of their origin and fate.

The *tip of the branch* consists of the two layers of cells which are found in other parts of the body wall, — the ectoderm and the mesoderm, as the cœlomic epithelium may, for brevity's sake, be called. The cells of the ectoderm at the extreme tip (Plate I. Fig. 6) are greatly elongated, forming a columnar epithelium. There are about 25 or 30 of the larger cells. They have a length of 28 μ to 32 μ, and a diameter of about 4 μ. They possess an ovoid nucleus averaging 5.7 μ by 2.6 μ, which lies in the middle of the cell but slightly nearer the cœlomic epithelium than the cuticula. It possesses a large nucleolus over 1 μ in diameter, which often appears stellate owing to the threads of plasma surrounding and proceeding from it and forming a nuclear network. As the figure shows, the plasma of the cell is filled with large, apparently deeply stained granules, some of the largest being over 0.6 μ in diameter. The coarser granules lie chiefly in the immediate vicinity of the nucleus, but are also found arranged in long lines at right angles to the surface throughout the greater part of the cell, becoming finer the farther they lie from the nucleus. A fine network can sometimes be made out between the large granules, but this appearance is more evident at the peripheral portion of the cell, where there are no large granules. At the outer and inner ends of the cells one finds large vacuoles, the largest of which are of about the same size as the nucleus; these become smaller the nearer they lie to the nucleus. In many cases the larger vacuoles are each seen to be partly filled by a body which stains slightly, and, as focusing determines, is more highly refractive than the plasma. Similar highly refracting, slightly staining granules are found in, and in fact often composing, the smaller "vacuoles." Owing to the fact that the deeply staining granules lie near the nuclei, and that the vacuolated and finely granular plasma lies more remote, there is a very marked deeply staining band occupying the middle of the ectodermal layer, and having about four tenths the thickness of the whole layer.

At the outer ends of the cells, and doubtless secreted by them, there is a cuticula about 1 μ thick. Its inner surface is sharply marked off from the underlying plasma; its outer surface is less sharp, and there are usually very minute particles of dirt attached to it (not represented in the figure). The whole cuticula forms in section a continuous band of substance, which stains deeply in Ehrlich's hæmatoxylin (but not at all in alum cochineal), and covers nearly the whole tip. Looked at from

the surface after staining in hæmatoxylin, it appears uniformly dark. The mesoderm of the tip is highly modified, and a description of it will be more instructive after I shall have described the normal cœlomic epithelium, as I shall do later.

Passing from the extreme tip towards β (Fig. 14), one finds the ectodermal cells gradually changing in form, size, and structure, and becoming slightly broader, and very much shorter. Their nuclei lie near the inner ends of the cells, possess a thick "nuclear membrane," and are more nearly spherical than those of the columnar cells, but of about the same size. They each possess one very large, centrally placed nucleolus, whose diameter equals and sometimes exceeds one third that of the nucleus, and whose outline is often somewhat stellate. Outside of the nucleus in the cell body there are fewer and fewer vacuoles as we pass from the tip, but the plasma is still coarsely granular, and here, as before, these stained granules surround the nucleus. It is now the regions between cells rather than those at the inner and outer ends which remain unstained, so that the cells are separated from one another by light spaces.

The mesodermal layer becomes somewhat thinner than at the tip, that is to say, its cells are flattened. The nuclei are elongated in the axis of the branch, and average about $4\,\mu$ by $2.2\,\mu$. They possess one spherical nucleolus, whose diameter is about two thirds of the minor axis of the nucleus. Small, clear vacuoles often with highly refractive spherical bodies are abundant in the cell protoplasm, which stains as a whole less deeply than does the ectoderm. Such highly vacuolated elements will be called *reticulated cells*.

If we study the *gemmiparous zone* at a stage considerably earlier than that shown in Figure 14, in fact at a stage in which a polypide is about to arise, we find an appearance of the layers represented by Plate I. Fig. 3. In such a region the ectoderm consists of cuboid cells about $7\,\mu$ high by $6.5\,\mu$ broad. The nuclei are large, nearly spherical, and vary in size from $3.5\,\mu$ to $6.0\,\mu$. The largest nuclei are those in the region from which a bud is about to arise (*ex.*). One in this region (to the right of *ex.*) is $6.5\,\mu$ by $6.0\,\mu$ in diameter, with a nearly spherical, eccentrically placed nucleolus of about $3.0\,\mu$ in diameter. This nucleus is the largest which I have found in the whole tissue of Paludicella, and the same is true of the nucleolus. From the examination of many regions from which buds are about to arise, I can assert that such regions always, in Paludicella, possess large nuclei and large deeply staining nucleoli. I shall have occasion to describe similar conditions elsewhere, and to point out the probable significance of these facts. The cell body possesses a highly granular, deeply

staining plasma; the inner ends of the cells, however, do not stain so deeply as the middle or peripheral portions.

The cuticula (omitted from Fig. 3, see Fig. 5) is usually somewhat different in appearance from that at the extreme tip. In section we can distinguish two layers: an outer, thicker, deeply staining layer, which is not continuous but appears broken into larger or smaller bits; and an inner, thin, non-stainable and highly refractive portion, from which the first layer is often slightly separated. This second layer is closely applied to the underlying cells, which doubtless secrete it. Looked at from the surface (Fig. 10, *a.*) the deeply stainable layer is seen to be broken into irregular polygonal pieces ranging from 2 μ to 17 μ in diameter and separated from one another by spaces ranging from 0 to 6 μ.

The mesoderm forms a loose epithelium, whose average width is less than that of the ectoderm (Fig. 3, *ms'drm.*). As a whole, moreover, it stains less deeply. In a portion of the gemmiparous zone, which lies about 180° from the budding region, the mesoderm has become so delicate a layer, if it exists at all, as not to be easily distinguishable. In the vicinity of the bud its cells have irregular outlines and extend out into the cœlom as though possessed of the power of amœboid movement. The nuclei are spherical or ovoid, smaller than those of the ectoderm, and on the whole have smaller nucleoli. The cell body is highly vacuolated. The vacuoles are not large and clear in outline, but whole regions of the cell body seem to be reduced to a non-stainable condition, and in some of these regions a fine network may still be observed.

The *proximal zone* (Fig. 14, γ to δ) is distinguished, soon after the first rudiment of the bud appears, by the diminished thickness of the ectoderm. The cells have become transformed from a columnar to a pavement epithelium. The nuclei are smaller, the nucleoli less prominent, and the cell body stains much less deeply. The cuticula is of two kinds, as before, but with this difference: the deeply staining outer part is less conspicuous, and the pieces are smaller and more widely separated. Looked at from the surface, we find an appearance like Figure 10, *c.*, in which the dark bodies represent the deeply staining cuticula. These pieces are much smaller than those of the gemmiparous zone, ranging from 0.6 μ to 9.5 μ in diameter, and separated from each other by spaces ranging from 0 to 13 μ.

3. Origin of the Polypide in the Terminal Bud.

Observation having shown that budding in Paludicella follows definite laws, we ought to be able to discover the place and time at which buds

will arise ; and it is necessary to do this in order to study the origin of the gemmiparous cells, and the changes which they undergo preparatory to an actual involution.

The study of tips of branches shows that the necks of the polypides of any branch all lie in one plane, and that this plane also includes the youngest polypide ; also that the youngest polypides always arise distad of the next older. Knowing these facts, our observations may be confined to a short line running from the neck of the youngest apparent buds to the tips of the branches studied. The time at which to search for incipient buds and the place in the line where they will be found is illustrated by Figure 7 (Plate I.). The youngest *developed* bud is one the axes of whose tentacles are approximately parallel to the axis of the branch, and whose brain cavity, *gn.*, is not yet constricted off from that of the œsophagus. The place of origin is near the tip, immediately beyond the point at which the ectoderm changes rapidly from a columnar to a pavement epithelium.

Figure 3 is from a section across the branch in the region of an incipient bud. I have already described the conditions of the cells of this region. Those near *ex.* are larger than the surrounding ones, and show signs of cell division both in the ectoderm and mesoderm. In both cases shown in the figure, the direction of division is such as will tend to increase the superficial area of the layer in which it occurs. The ectoderm seems to be the most important layer of the two in the process of invagination which is about to take place. I think one is led to this conclusion if one considers a folding of an epithelium to be due to an increase in the area of the epithelium within a certain circumference without a correspondingly great increase in the circumference itself. Such a conception implies, first of all, mutual pressure of the cells of the invaginating epithelium. The cells of the mesodermal layer do not seem to be under mutual pressure ; in some cases they are barely in contact. The cells of the ectoderm are evidently closely applied, and probably, therefore, under mutual pressure.

The one case of cell division which is occurring in the ectoderm is at the inner end of the cell. In fact, the centre of the nuclear plate is much nearer the deep end than are the centres of the adjacent nuclei. The effect of this division is to increase the area on the inner surface of the ectoderm more than that on the outer, as appears from a study of the sections shown in Figures 4 and 5. In Figure 4 certain cells lie already below the *niveau* of the surrounding ones, very much as though they had moved downward on account of this being the direction of least resistance. A later stage of this process is shown in Figure 5. Here the

nuclei are already arranged in a deep saucer-shaped layer. The transition to the U-shaped arrangement of Figure 37 (Plate IV.), in which the invagination of the inner layer of the bud is completed, is not a difficult one to understand. It is to be observed, however, that the folding is of such character that it can hardly be termed a typical invagination. Comparing Figures 4, 5, and 37, it appears rather to be of a type somewhat intermediate between typical invagination and typical ingression. The cavity of the bud first arises through a rearrangement and reshaping of the cells of the inner layer of the bud. At this stage the nuclei of the invaginated region stain very deeply, and have large nucleoli.

Figure 21 (Plate III.) shows the condition of the bud at this stage as seen in longitudinal section. The proliferation which gave rise to the rudiment of the bud is shown, by a comparison of Figures 37 and 21, not to have been confined to one point, but to have occurred along a line, so that the resulting bud is boat-shaped, and not cup-shaped. The whole mass is therefore bilaterally symmetrical. Even at this early stage one can distinguish a difference in the form of the bud at the anal and oral ends. At the oral end (*Or.*) the bud passes more abruptly into the body wall than at the anal end. Later, this feature becomes more marked. This is an indication of a fact for which later stages will bring better evidence: that the formation of the bud proceeds from the oral towards the anal end, and that the increased length of the bud that one finds in the stage represented by Figure 22 is due to growth at the anal end.

4. Origin and Development of the Lateral Branches.

The first lateral branch appears as a prominent protrusion of the lateral walls of an individual of the primary branch when the ganglion of that individual has already nearly closed, and when the bud of the next younger individual has attained a stage somewhat later than that shown in Figure 37. The zone in which the lateral buds arise lies about midway between the neck of the median polypide and the tips of its tentacles at this stage. The place of appearance in this zone is approximately 90° to the right or left of the neck of the polypide of the median individual. In one case measured, however, that shown in Figure 20 (Plate II.), the centres of the two lateral buds seemed to be unequally distant from the neck of the polypide, and each over 90° from it (approximately 100° and 110° respectively. (Compare page 3.)

A cross section of the branch through the region in which the lateral bud is arising shows that the condition of the body wall at the bud is quite different from that of the rest of its extent. Figure 19 represents

a longitudinal section of a portion of the body wall passing through the non-budding region. The wall seems to consist of one layer only of cells, and a fine, non-stainable cuticula. This layer of cells is the ectoderm, for it can be traced directly into the outer layer of the tip. The mesodermal layer is not represented in the region from which the figure was drawn, but I believe it is not entirely absent from this part of the individual, for occasionally extremely flattened cells, spindle-shaped in section, may be seen lying inside of the ectodermal layer, quite sharply marked off from it by a distinct line. Further evidence of the existence of two layers is found in the fact that one occasionally sees in the flattened body wall two nuclei lying together, one nearer the cœlom than the other. The cells of the ectoderm are seen to be very much flattened (average 2.5 μ), and their nuclei are widely separated (35 μ). The nuclei are oval, and rather smaller than those near the tip. They possess a single, rather large nucleolus, which does not stain intensely. The cell protoplasm stains very little. The cuticula is about 0.5 μ thick.

If we study the body wall in the budding region, when the latter is first indicated on the surface by a marked protrusion of the outline of the zoœcium (Plate II. Fig. 15), we find that this protrusion is due to an elongation of cells. There are about twenty-two cells in this section, which are more or less thickened. Since the section figured passes through the centre of the circular thickening, and is about one sixteenth the diameter of the circle in thickness, it follows that there are over 250 cells of the ectoderm which have already at this stage become somewhat enlarged previous to evagination. The highest of these cells are the central ones, of which the largest is 22 μ high. The largest nuclei are 4 μ by 6.3 μ, which approximates the size of those in the gemmiparous region (page 6). They are placed nearer the cœlomic epithelium than the exterior, are nearly spherical, and each possesses one large nucleolus and a quite apparent network with deeply stainable nodal points. The cell body is stained as a whole rather deeply by Ehrlich's hæmatoxylin, but particularly around the nuclei. The outer parts of the central cells, however, are stained very little, and the deep ends of some of the lateral cuboid cells not at all. The network of plasma contains only fine granules, and these seem to lie in rows parallel to the long axis of the cell. The structure of the outer-layer cells, at a somewhat earlier stage, is shown in Figure 18, under a higher magnification. The network is very apparent in these large spherical nuclei, and the plasma of the cell is seen to contain coarse granules, which lie near the nuclei and stain deeply.

While the cuticula of Figure 18 is seen to be that of the normal body wall in this region, that shown in Figures 15 and 16 appears under the microscope after staining in hæmatoxylin to be of two distinct kinds: (1) that outside of the central region, which is highly refractive and not at all stained; and (2) that which lies immediately over the central elongated cells of the bud, which is also highly refractive but stains deeply. In fact, the central cuticula resembles in every way that already described for the tip of the branch, and shown in Plate I. Fig. 6. Moreover, it has other points of resemblance to the latter. It does not stain at all in alum cochineal; the outer boundary of the branch is often uneven at this place (Fig. 16); and particles of dirt are often found adhering to it, while the rest of the cuticula is comparatively free. The difference in staining properties of the central and lateral cuticulas indicates that the former undergoes with age a change in its chemical properties; the irregular outer boundary and the adhesion of dirt particles seem to indicate that the newly formed cuticula is viscid. The mesoderm of the stage of Figure 15 consists of a single loose layer of subspherical cells of the two kinds already noticed, reticulated and non-reticulated. The series of Figures 18, 15, and 16 shows the behavior of columnar cells in the formation of a typical outfolding as distinguished from the slipping in of cells to form the polypide (Figs. 3, 4, and 5).

In stages later than that of Figure 16, the tip of the branch becomes further removed from the body wall of the median branch. The cells at the tip always retain their elongated columnar condition. A polypide is soon formed on the upper part of the body wall immediately behind the tip, exactly as in the case of the median branch. A septum is very early formed, cutting off the lateral from the median individual, and the lateral secondary branch becomes the median primary one of new individuals (Plate VI. Fig. 58).

We have already traced out the origin of the polypide of the median branch from the mass of cuboidal cells near the tip; it remains to determine whether the cells which give rise to the lateral branch can be traced directly back to the cuboidal cells of the tip, or whether they have arisen from the flattened epithelium of the general body wall and secondarily acquired their plump "embryonic" character.

Figure 18 (Plate II.), to the cellular conditions of which I have already referred, shows an early stage of the lateral branch, and Figure 20, *gm. l.*, shows on a smaller scale the different cellular conditions in the body wall in the region of two lateral buds which are yet far from showing external signs of evagination. The cells are cuboid and much higher than

those of the adjacent body wall. Have they been so ever since they were derived from the tip, or have they secondarily become so ? I believe that these cells have never been flattened pavement epithelial cells, for the following reason. All ectodermal cells of the body wall near the tip are cuboidal; if these cells have only secondarily acquired this form, they must have passed through a stage in which they were flattened epithelium. Now, if these cells could be distinguished by greater thickness from the cells of the surrounding body wall, at a time at which the latter cells had only just begun to emerge from the cuboidal condition to become differentiated into the pavement epithelium of the body wall, it would follow that, even though they had secondarily increased in size as a result of an impulse preparatory to evagination, and even though they would have been at a stage only a very little earlier indistinguishable from the other cells of the body wall, yet they would never have passed in this case through a flattened condition, because at a stage only a very little earlier the whole body wall was composed of cuboid cells.

The conditions which I have set as the criterion of our problem are fairly realized in Figure 17, which represents a portion of the body wall of a median branch which extends from the gemmiparous region above to the thickened body wall of the nascent lateral bud below (*gm. l.*). It will be seen, by a comparison of the body wall of this region with that shown in Figure 19, which is taken from the same individual farther from the tip, that even the most differentiated part of the body wall of Figure 17 is in a relatively indifferent condition as compared with the pavement epithelium of the ectoderm of Figure 19, in which the mesoderm, indeed, has become so thin and insignificant as scarcely to be visible. We may, therefore, maintain that the ectodermic cells of the body wall have only just begun to lose their cuboidal condition to become pavement epithelium, and therefore conclude, in accordance with the argument just presented, that the cells of the lateral bud (*gm. l.*) have never passed through a stage in which they were flattened epithelium. It is evident, also, that the *Anlage* of the second lateral bud is also derived from near the tip, because, as in Figure 20, we find two lateral regions of cuboidal cells.

5. Development of the Body Wall.

It is, of course, almost impossible to gain direct evidence upon the place of origin and method of development of the body wall, and one is therefore forced to the collection and weighing of circumstantial evidence. Braem ('90, pp. 127, 128, 131) believes that the body wall (the

cystid, in Nitsche's sense) has a double origin in Paludicella: "Ein Theil des Cystids zwar vor dem Polypid, ein anderer aber erst später angelegt und zwar aus der polypoiden Knospe selbst entwickelt wird." The part developed from the bud of the polypide is the elliptical region of the body wall, whose main axis lies in the sagittal plane and which has the neck of the polypide at the distal focus and the attached ends of the retractor muscles and the parietovaginal (or pyramidal) muscles lying in the proximal circumference, — the greatest part of the ellipse thus lying oral of the atrial opening. The evidence for this conclusion Braem finds in the following facts, which my own observations confirm: "The great retractor first appears in the angle between the oral part of the polypide bud and the cystid wall [cf. Figs. 23, 24, *cl. mu. ret.*]; then its cells gradually become elongated, and as its point of origin retreats farther and farther from the polypide, it finally appears as a bundle which joins a point lying between the mouth of the polypide [neck of the polypide] and the inferior septum with the pharynx, and, as I believe, also with the cardial part of the stomach" (p. 125). Compare the muscles at the left end of Plate I. Fig. 8. Further on he says: "Die Parietovaginalmuskeln [pyramidal muscles] erscheinen an der Knospe zuerst in Form zweier seitlichen Leisten, in welchen die einzelnen jugendlichen Fasern senkrecht zur Längsaxe verlaufen. Indem sich alsdann lateral von der Knospe das Cystid durch Neubildungen erweitert, werden die Fasern verlängert und die beiden Bündel treten in Flügelform deutlicher zur Rechten und Linken der Mündung hervor. Ihr Ursprung an der Cystidwand rückt nun von der Mündung immer weiter ab und gelangt schliesslich auf die gegenüberliegende Seite, wo er anal und lateral seinen definitiven Platz findet." Compare Plate I. Figs. 7–9, *mu. pyr.*, and Plate VI. Fig. 63, *mu. pyr.* From these observations Braem ('90, pp. 127, 128) concludes: "So scheint es sicher, dass auch hier ein grosser Theil des definitiven Cystids, das ja zum anderen Theil schon vor der polypiden Knospenanlage entwickelt war, aus dem Material dieser letzteren hervorgeht. Das folgt namentlich aus der Art und Weise, wie sich die Muskeln bilden. . . . Auch hier würde, wie bei den Phylactolæmen, oral vor der Knospe nach dem Retractor hin, ein grösseres Gebiet der Leibeswand der Knospenanlage entstammen, als seitwärts und hinten."

An analysis of the facts has led me to conclusions differing somewhat from those of Braem; namely, that all or nearly all of the cells of the body wall (cystid) are derived from the tip of the branch or from the immediate descendants of cells so derived. The number of cells contributed to

the formation of the body wall by the neck of the polypide is much smaller than Braem has suggested, and probably insignificant in amount. The retreat of the points of origin of the retractor and parietovaginal (pyramidal) muscles may be in part accounted for by the normal growth in area of the body wall, and in part by the actual movement of the point of origin with reference to the cells of the body wall. These conclusions rest upon the following circumstantial evidence.

Owing to the small number of cells in the body wall at the tip, and the comparatively slow growth of the cystid, karyokinetic figures are much less frequent than in the polypide. Quite a long search has therefore not afforded cases enough to enable me to draw any perfectly satisfactory conclusions as to just where, and where only, growth was taking place. I have, however, seen nuclear division occurring in the elongated cells of the extreme tip, rather more abundantly in the cuboidal cells between the extreme tip and the gemmiparous zone, and most abundantly in the gemmiparous zone, but here evidently having to do with the origin of the polypide, muscle cells, etc. Proximal to the gemmiparous zone, I have noted few cases of nuclear division excepting about the neck of the polypide. It seems probable that the cells of the tip of the branch are not to be regarded as forming a differentiated organ whose elements rarely divide, but as quite capable of adding new cells to the body wall. On the other hand, there is by no means a *Scheitel* in the botanical sense, but the cells added to the body wall continue for a time to divide vigorously, and finally give rise to the polypide, to the *Anlage* of the lateral branches, and to the body wall. The cells belonging to the proper cystid then cease to divide rapidly.

I have already shown how the cells of the tip secrete a cuticula, which becomes gradually replaced by a second cuticula secreted beneath it as the body wall attains its adult dimensions. It appears as though the first cuticula were secreted by the cells of the tip *only*. This being so, since the area of the body wall increases, this first cuticula must either stretch to cover the enlarged area, or else it will fail to cover it and appear as isolated patches upon the body wall, and these isolated patches will become more and more widely separated as the area of the body wall increases. This latter condition seems to be the one realized in this case. The presence of the old cuticula is easy to demonstrate, since it stains deeply in hæmatoxylin ; and it may be easily distinguished from that formed later, for with the same reagent this stains not at all. Figures 6, 11, 12, and 13 show different appearances of the cuticula at different parts of the body wall. At the extreme tip (Fig. 6) there is a continu-

ous deeply stained band of cuticula. In Figure 11 it no longer appears quite homogeneous, but is darker at some places than at others. The ectoderm is here composed of cuboidal cells. At a later stage of development the ectodermal cells have become very much flattened. A thin, unstainable, more deeply lying cuticula has already begun to form, and the outer deeply stainable cuticula is seen to be broken up into bits. Figure 13 is from the adult body wall. The ectoderm is flattened. The inner cuticula has attained a great thickness, and the outer cuticula is represented by only a few deeply staining patches. One attains a similar result by studying the surface of a stained individual. Figure 10 shows the condition of the outer cuticula at intervals along the same branch from the gemmiparous region *a* to a nearly adult region, *d*. The bits of cuticula become more and more widely separated and smaller, as I have already described in detail on page 7. Here, then, we have not merely an interesting case of replacement of one cuticula by another to meet the needs of the enlarged body wall by a method which has no parallel, so far as I know, in any other group of animals, but for the specific purposes of our problem *a criterion of growth of the body wall* quite as satisfactory as karyokinesis, and much easier of application.

Let us apply this criterion in our attempt to answer the question, Is that portion of the body wall lying between the neck of the polypide and the points of origin of the pyramidal muscles (Plate VI. Fig. 63, *b*–*a*, *b*–*c*) derived wholly from the neck, or is it merely the result of interstitial growth of that part of the original cystid which was preformed in the neck region? If the first condition is true, we should expect to find no indications of the outer cuticula secreted by the tip of the branch; if the second, we should expect to find the outer cuticula broken into bits, and underlaid by the inner lately formed cuticula. Figure 63 shows clearly the deeply stained outer cuticula here separated into bits, and, to my mind, thereby proves that this part of the cystid has had an origin similar to that of the rest of the body wall. Moreover, a comparison of the portion of the section figured with the remainder (and this comparison has been made on many sections from several individuals) shows that the parts of the cuticula about the neck are indeed rather smaller and farther removed from each other than at the opposite side of the branch; but the difference in this respect is not very marked, and may well only signify that there is a more rapid growth of the body wall in the vicinity of the neck of the polypide than at the opposite side.

But how then do the points of origin of the pyramidal muscles come

gradually to move away from the neck of the polypide at which they arose, in order finally to lie so that the muscle fibres are nearly parallel? If the points of origin remain fixed with reference to the surrounding cells, they can hardly come to lie *absolutely* closer together, but only *relatively* so by growth of the body wall between these points and the neck. If, however, we find that in older individuals the points of origin are not only relatively but absolutely closer together, we are driven to the conclusion that these points move relatively to the surrounding cells. To decide whether the points of origin come to lie closer together behind the neck *absolutely* or only *relatively*, I measured cross sections of four individuals through the region of the neck in which the muscle fibres showed evident differences of length, and therefore of age. I may preface a table of these measurements with the statement that the muscles first appear plainly differentiated at a stage when the polypide is well formed (Fig. 7, *mu. pyr.*), and that the growth of the body wall in circumference is not very considerable after this time. The numbers indicate measurements in micra:—

	No. 1.	No. 2.	No. 3.	No. 4.
Distance on periphery between origins of muscles, atrial side	150	154	187	260
Distance on periphery between origins of muscles, abatrial side	297	286	264	220
Total length of periphery	447	440	451	480

The distance on the "atrial side" signifies the distance measured over *a*, *b*, *c*, Figure 63 (Plate VI.). The length of the remainder of the section is the distance on the "abatrial side."

From these measurements it appears that the "origins" of the pyramidal muscles approach each other absolutely, — a condition which Braem's hypothesis cannot explain, and which can be reasonably interpreted, it seems to me, only by assuming, however unique and difficult of conception such a condition may be, that the points of origin move relatively to the surrounding cells of the body wall. (Compare also the movement of parietal muscles referred to on page 29.[1])

It is not necessary to assume that the increase in extent of the body wall after the polypide is first formed is due to the addition of cells from

[1] Professor Mark has called my attention to a discussion of the movement of the fixation-point of a muscle in Mollusks by Tullberg ('82, pp. 26, 27, 44). This author says that he has undertaken no special investigation of the method of migration, but concludes that this motion must result from the absorption of the inner muscle fibres as new ones are formed on the outside. I do not find any evidence of such a process in Paludicella.

the neck. The change in form of the ectodermic cells from a columnar to a pavement epithelium must alone cause a great increase in the extent of that layer. Some measurements that I have made seem to me to prove that the area of the body wall does increase greatly, even outside the region whose growth Braem attributed to the addition of cells from the neck of the polypide. Thus, in one case, the distance from the distal end of the polypide bud, which becomes the neck of the adult, to the point of origin of the young retractor muscle was 0.17 mm.; from the same point to the septum separating the young individual from the next older was 0.27 mm. In the next older individual, from the neck to the origin of the retractor muscle was 0.72 mm.; from the neck to the septum was 2.0 mm. Thus assuming that the older individual passed through a stage exactly equivalent to that in which we find the younger, the distance from the neck to the origin of the retractors has increased 0.55 mm., and from the origin of the retractors to the septum 1.18 mm. The first distance is that in which Braem has assumed the body wall to grow by additions from the neck of the polypide, and this assumption was apparently made to account for the increase in extent of this region; but the area between the origin of the retractors and the septum, which is outside the region to which additions such as Braem contemplates could have been made, has grown in this case very considerably more in extent. This case is not a typical one, however, for we rarely find the distance from the origin of the retractor to the septum to be so great. In general, from observation of a number of cases, I should say that in the adult the distance between the neck of the polypide and the origin of the retractors, is to the distance between the latter and the septum about as 5 : 4, and that therefore the growth of the first region is slightly greater than that of the second. From the fact, however, that the cells around the neck of the polypide for a long time retain a somewhat embryonic character, and may quite frequently be seen in division, this was to have been expected. The conclusion which I draw from this last series of conditions is, then, that it is unnecessary to suppose the addition of cells from the neck of the polypide to account for the fact that the origin of the retractors is carried backward from the polypide. Normal growth of the body wall, such as occurs elsewhere, is quite sufficient to account for it.

To recapitulate. That portion of the cystid lying in the vicinity of the neck can hardly be derived from the neck alone, for the cells still show adhering to them the cuticula which they derived from the tip of the branch. It is not necessary, in order to account for the movement

of the origins of the muscles away from the neck, to suppose that the circumcervical region is derived in that way; for (1) the origins of the pyramidal muscles actively migrate away from the neck to a certain extent, and (2) the normal growth of the body wall is sufficient to account for the carrying backward of the origin of the retractors.

From the facts already gained it seems clear that the ectocyst (cuticula) is first formed at the tip, and then, to meet the wants of the growing colony, this is replaced later by a cuticula of different chemical composition, which becomes thicker as the body wall grows older. At a late stage we find a separation of the thick cuticula itself into two layers, of which the outer one is much the more highly refractive.[1] (Plate II. Fig. 13; Plate III. Figs. 26, 29.)

6. Development of the Polypide

We have already (pages 8, 9, Figs. 5, 14, 37) seen how the foundations of the polypide are laid by the ingression of cells of the outer layer of the body wall pushing before them the mesoderm, and how, finally, those cells arrange themselves in a boat-shaped mass to form the inner layer of the bud (Plate III. Fig. 21), which possesses no actual cavity, and is constantly separated from the external world by the ectoderm which remains behind to form the neck of the polypide. Even when a cavity is formed later, it does not communicate with the exterior until the permanent atrial opening has arisen. The earliest differentiation in the bud is, as mentioned by Allmann ('56, p. 36), the formation of a cavity which is to become that of the atrium. This cavity is first formed at an early stage as an extremely slight fissure in the midst of the inner layer. Figure 22 shows a longitudinal section of this stage. Cell division is taking place throughout the whole mass, but especially at the neck of the polypide, *cev. pyd.* The position of the cavity is represented by the central non-nucleated space, and this gives rise, as the later history of development shows, to the atrium and the pharynx.

Figure 23 represents a stage which is doubtless of short duration, for I have found it only twice. The bud is much more developed at the

[1] Such a two-layered condition of the cuticula was long ago described by Reichert ('70, pp. 265, 266) for Zoöbotryon. He distinguished "eine *äussere, festere,* stärker lichtbrechende und sprödere Schicht und die *innere weichere.*" Realizing that the "ectocyst" or cuticula undergoes many changes in form, — formation of lateral buds, of septæ or communication plates, and increase in size of the stolon, — he suggested, without having observed the process, that probably during these changes the more rigid outer layer disappeared and was replaced by the inner softer one.

anal end than in the last stage, and there is a second cavity below the atrium, from which it is separated by a line of nuclei. This is plainly an early stage in the formation of the *alimentary tract*, which thus first appears at the anal side of the bud, as in Phylactolæmata, and is progressively formed towards the oral end. An appearance similar to the one figured would be given by a slightly oblique section of a later stage; but this section is strictly sagittal, and no trace of the lumen appears in adjacent sections. I have found a similar condition in a series of longitudinal sections at right angles to the sagittal plane of the bud (Plate IV. Figs. 39 and 40). Figure 39 shows that the atrio-pharyngeal cavity is first developed at the anal end, and in Figure 40, which is three sections (about 15 μ) below Figure 39, the anal end only of the alimentary tract is formed. It is worthy of notice that the cells of the mesodermic layer of the bud are often greatly vacuolated at this stage, as in Figures 39 and 40, *vac.* Braem ('90, p. 126) says of this stage: "Die der Resorption dienenden Darmabschnitte, Magen und Enddarm, werden gemeinsam angelegt, indem auf jeder Seite der Knospe eine Längsfalte die Wandungen nach innen und gegen einander zu einbiegt, worauf die benachbarten Theile des inneren Blattes verschmelzen und so durch eine Art Abschnürung das primäre Knospenlumen in den vorderen Atrialraum und die hintere Darmhöhle getrennt wird." While I thoroughly agree with this statement, the additional fact of the formation of the tract *progressing from the anal towards the oral end* is interesting, in that it shows that the process of formation of the organ in Paludicella is fundamentally similar to, although differing slightly in detail from, that of the Phylactolæmata. Figure 24 shows in sagittal section a still later stage in the development of the alimentary tract. A cross section of this stage is seen in Figure 30 (Plate IV.), in which the separation of atrial and gastric cavities is demonstrated. The inner layer of the bud is here seen to be separated from the ectoderm by a distinct line, and, to a certain extent, even by the mesoderm. The distal (oral) part of the cavity of the alimentary tract next becomes considerably enlarged to form the stomach (Fig. 25). The outer layer of the bud, *ms'drm.*, penetrates between the stomach and the atrium, and a depression is formed at the bottom of the atrial chamber which will give rise to the œsophagus. Even at this stage the œsophagus is not in communication with the stomach, but their cavities are separated by two layers of cells of the inner layer of the bud. These two layers become those of the cardiac valve (Plate IV. Fig. 36, *vlv. cr.*). By a further comparison of Figures 25 and 36 it will be noticed that, whereas in the earlier stage, as in Endoprocta, there is no cœcum to the

alimentary tract, in the later stage the cœcum has already begun to form, as in Phylactolæmata, by an outpocketing of nearly the whole of the lower wall of the stomach. (Compare also Plate I. Figs. 7, 8, and 9.)

Very soon after the establishment of the alimentary tract, and between the stages shown in Figures 24 and 25 in sagittal section, there begin to appear organs which have a very considerable phylogenetic significance; namely, the lophophoric ridges, ring canal, and tentacles.

The *lophophoric ridge* is a fold which surrounds the mouth, and from which at intervals tentacles arise. The ridge, however, arises before the tentacles. The general position of the ridge, as well as its method of origin, may be learned from an inspection of a series of sections of the age of those shown in Figures 31–34. In a section lying near the oral end of the bud (Fig. 33), one finds two spaces, — a lower, which is that of the stomach, and an upper, the œsophagus and atrium. This upper space is broader above than below, and the cell layer which lines it is thick below, but above, or nearer to the body wall of the budding individual, it is thinner. The transition from one condition to the other is quite abrupt, and is marked by a salient curve (*loph.*). In a section near the anal end of the bud (Fig. 31), it will be seen that here too the inner layer is thick below and thin above. The characters mentioned are still more strikingly shown in the median section, Figure 32. That the differences in thickness of different parts of the inner layer are recently acquired modifications of an earlier simpler condition is indicated by comparing Figure 32 with Figure 30, which is from a younger bud. The series of points (*loph.*) of transition from thick to thin epithelium forms on the reconstructed polypide a curved line, convex above. This line is the ridge of the young lophophore (compare Fig. 25, *loph.*). I have said that the lophophoric ridge arises before the tentacles. The evidence for this assertion is found in a series like that referred to above, where, although the ridge exists along the entire side of the atrium, one finds nascent tentacles in the middle region only (Figure 32, left hand).

As Figure 25, of a later stage than Figures 31–33, shows, the lophophore curves downwards rapidly at the anal end, so that it here lies at right angles to the axis of the rectum, but does not extend at all beyond the anus. Orally, there is in the median plane only the slightest trace of the lophophoric ridge. By the formation of this ridge in the wall on each side of the atrial chamber, the original atrio-pharyngeal cavity has become separated into two regions. The space lying within or below the ridge forms the pharynx and the intertentacular space; that lying with-

out and above, the atrium of the adult. (Plate III. Fig. 25; Plate IV. Fig. 32, *atr.*) Since the lophophore curves rapidly downward to the anus and does not extend behind it, the act of cutting off the lower part of the atrio-pharyngeal cavity from the upper (atrium proper) does not continue behind the anus, which therefore opens directly into a part of the atrium. This part has the form of a compressed funnel, and is bounded behind and laterally by the kamptoderm, and orally by the hinder ends of the lophophoric ridges, and also, since the latter do not meet in the median plane, by the pharyngeal cavity. Thus it has come about that the anus, which at first opened into the common atrio-pharyngeal cavity of the bud, has now, in the separation of the two regions, come to lie near their point of division posteriorly, but to open distinctly into the atrial cavity. The more pronounced separation of the part of the atrial cavity into which the anus directly opens from the remainder of the atrium takes place much later, and will be described further on.

In Figure 33, the *ring canal* (*can. crc.*) is seen to be already formed. At this stage it is found on one side only, the left, if one looks at the polypide from the tip of the branch. It occurs in only four sections (each 5 μ thick), being found on the next section behind Figure 33, and on two sections nearer the oral end. At its oral extremity, it terminates blindly as a thickening of the outer layer of the bud; at its anal end, one sees cells of the outer layer extending out partly over the canal, but failing to enclose it; in the next section the mesoderm is undisturbed. In similar sections of an older polypide (corresponding in age approximately to Plate IV. Fig. 35), the canal is found on both sides, and near to the oral end, but at about the middle of the series (cf. Fig. 35) it is found to open again into the body cavity. I therefore conclude that the ring canal makes its first appearance at the base of the lophophore in a region just oral of the middle of the polypide. Exactly how it arises, whether by a growing together of the lips of a shallow furrow formed from the mesodermal layer, or by the formation of a pocket, which, elongating, penetrates between the inner and outer layers of the polypide at the base of the nascent lophophore, I have not been able to determine. Two facts induce me to believe that the later formation of the canal oralwards results from the penetration of a sac-like mass of mesodermal cells between the two layers of the polypide at the base of the nascent lophophore. One usually finds, (1) as in Figure 33, *can. crc.*, a double mesodermal wall between the lumen of the canal and the cœlom, and one layer between the former and the inner layer of the bud; and (2) at the oral blind end of the ring canal a number of loose cells

(occasionally dividing) representing the blind end of the pocket and lying between the inner and outer layers, both of which are intact.

Braem ('90, p. 50) describes the formation of the ring canal in Phylactolæmata as taking place in the manner just suggested for Paludicella. His studies were made, he says, preferably on statoblast animals. Nitsche ('75, p. 358) concluded that in Phylactolæmata the ring canal was first a furrow, whose lips fused, and my own study ('90, p. 129) has led me to the same conclusion. Since reading Braem's account I have looked over some of my own sections of Cristatella again. Certainly the process is not so clear in the buds of the adult colony as in the statoblast embryo which Braem figures. Nevertheless the series of sections ('90, Plate IV. Figs. 33–38) given as evidence of my statement still seem to me capable only of the conclusion I drew from them. Perhaps the processes may be different in detail in the two cases; certainly the two explanations are not fundamentally dissimilar.

The ring canal being established in the oral part of the polypide, it grows forward, as I have said, and, secondarily, the canals of both sides meet in the median oral line and their lumina become confluent (Plate VI. Fig. 52, *can. crc.*). From what has already been said, it is clear that the lateral parts of the ring canal are not now continuous with each other behind. They become so only after the formation of the tentacles.

The *tentacles* arise upon the lophophoric ridge at a stage a little later than that represented in Plate IV. Figure 32. At the stage represented by Figure 35, however, the tentacles have begun to form, as indicated by the fact that in the series from which this figure was taken the fold into the upper part of the atrium appears now deep, now shallow, according as the section passes through the length of a young tentacle, or only through the lophophoric ridge between the tentacles. The position of the section (Fig. 35) is about the middle of the series, corresponding to Figure 32.

By a comparison of Figure 35 with Figure 32 in respect to the tentacles, it will be apparent, first of all, that the lophophoric ridge itself has been heightened and that this heightening has been effected, not by a deepening of the fold existing in Figure 32, the lips of the fold remaining quiescent, but by a movement downwards of the outer lip (*) of the groove which is to form the ring canal. The movement is of course accompanied by an increase in the length of the kamptoderm, *kmp. drm.* This growth of the lophophoric ridge naturally does not result in making the tentacles project farther above the ridge. Their elongation must

take place quite independently of the former's. The lophophoric ridges have now become elongated folds lying upon the right and left of the polypide, which at this stage has a very compressed appearance (Plate IV. Fig. 41). The folds occupy the position of the ridges, and therefore do not lie throughout their whole extent in one plane, but oralwards are nearly parallel to the body wall (Plate III. Fig. 25), analwards trend nearly at right angles to it. It results from this fact, that one cannot see the anal tentacles when looking at the polypide from the side of the body wall to which it is attached. Figure 41 (Plate IV.) shows also that no tentacles have yet made their appearance at the oral ends of the two lophophoric ridges.[1] The tentacles are here seen to be arising in two long rows, and so that those of one row are placed opposite the intertentacular spaces of the other. There are six tentacles in each row. The rows are not continuous with each other oralwards or analwards.

The separation of the atrial and oral cavities, begun by the first formation of the lophophore, is, now that the tentacles have arisen, much more pronounced. Other changes now occur in this region, which produce an extensive modification in the form of the polypide.

One of the first of these changes is the close approximation and finally fusion of the anal extremities of the lophophoric ridges oralward of the anus. A stage in this is shown in Figures 43 and 44 (Plate V.), which are sections in the position of the lines *43*, *44*, of Figure 25 (Plate III.), but through a slightly older polypide than that represented by Figure 25. The section shown in Figure 43 passes across the rectum, grazes the outer lip of the ring groove of the anal tentacles, and finally cuts, nearly longitudinally, one of the middle tentacles of the row. The two lophophores are not yet completely fused in front of the rectum. In Figure 44 (compare Plate III. Fig. 25, *44*) this break in the continuity of the lophophore is more prominent.

By the completion of the union of the lophophores in front of the anus, the rectum is quite cut off from communication with the intertacular space. It now opens only into the thin-walled, funnel-shaped depression of the atrial cavity.

Pari passu with this operation the stomach and rectum are being more completely separated from the pharyngeal cavity by the penetration of a double layer of mesoderm between these regions from each side, and a fusion of the corresponding layers of the two sides. Finally, the

[1] Compare Plate IX. Figure 77, which is a superficial view of the young lophophore from Flustrella, in which the process is similar to that in Paludicella, only the down curving of the anal tentacles occurs later than in the latter case.

walls of the stomach and pharynx become separated from each other by a part of the cœlomic cavity, as in Plate IV. Figure 36. This process of separation of the alimentary tract proceeds analwards, and finally the rectum is far removed from the œsophagus.

The anus thus comes to lie farther outside of the anal tentacles. Finally, the ring canal, which is formed progressively farther and farther analwards, follows the fusion of the anal ends of the lophophores, and thus completes the canal behind the œsophagus. (Plate IV. Fig. 36 ; Plate VI. Fig. 53, *can. crc.*)

The anal part of the ring canal is doubtless not merely a groove, but a tube ; but the ring canal is not closed at this, and probably not at any stage throughout its entire extent, for in Plate VI. Figure 52, two sections below Figure 53, an opening is shown to exist on each side (at *can. crc.*), putting the cavities of the ring canals and the cœlom into communication with each other. These openings lie at the sides of and slightly above the ganglion (*gn.*, Fig. 52) ; a position exactly comparable with that of the openings in the ring canal of Phylactolæmata, which leads from the cœlom into the lophophoric arms on the one hand, and into the circumoral part of the ring canal on the other.

By a comparison of Figure 41 (Plate IV.) with the sections shown in Figures 60–62, it will be seen that the row of tentacles has undergone a change of form : from being laterally compressed, it has become circular. This change of form has not resulted from an increase in the number of the tentacles, for at the stage of Figure 41 there are six tentacles on each side already formed (the sixth not visible), and there are in front of the mouth spaces already reserved for the two additional tentacles. There are also, probably, two nascent tentacles at the anus, although these are little developed, making a total of 16. In Figure 61 there are only 15 tentacles ; moreover, the actual diameter of the tentacular corona in the sagittal plane is less than at the earlier stage of Figure 41. This change of form is perfectly normal, all young polypides having tentacles arranged in two parallel rows, and adult polypides having a circular lophophore.

These changes in the form of the tentacular corona are correlated with important changes in the direction of the axes of other organs. These changes may be understood by comparison of Figures 25 and 36, together with the assistance of Figures 7–9, all of which are oriented in the same manner. In Figure 25 the points fixed by the cardiac valve (*vlv. cr.*) and anus (*an.*) lie in a line which is approximately parallel to

the body wall. In Figure 36 the line passing through the same points makes an evident, but not very large, angle with the body wall. This line has undergone, then, a slight change of position only. The axis of the anal tentacles lies in both cases nearly parallel to the body wall, and so does the neural wall of the pharynx. The oral tentacles, on the contrary, whose axes in the earlier stage are directed perpendicularly to the body wall, lie in the later stage with their axes parallel to the wall; and the base of the lophophore, which in the earlier stage trended at its oral end parallel, at its anal perpendicular to the body wall, in the later lies throughout its whole extent in one plane perpendicular to the body wall. The axes of the oral tentacles have rotated through an angle of nearly 90° relatively to most of the other organs of the polypide. The cause of this rotation must evidently be sought in unequal growth in different parts of the polypide. A comparison of the length of the kamptoderm on the anal side in Figures 25 and 7 indicates that it has grown more in length than on the oral side. This excessive growth would tend to rotate the line *vlv. cr.* — *an.* to a position perpendicular to the body wall. Since this rotation has not occurred to so great an extent as was to have been expected, we must look for a compensating growth on the oral side of the polypide, between *vlv. cr.* and the neck of the polypide, which shall be nearly equal to the excessive growth of the anal kamptoderm, and which must be *outside* of the oral kamptodem. These conditions of location are fulfilled only by the oral wall of the œsophagus, and it is by change of position and growth of this wall that the extension of the anal kamptoderm is nearly compensated for on the oral side of the polypide. By this growth in the wall of the œsophagus the oral part of the ring canal has been brought to lie over the anal part, the sagittal diameter of the tentacular corona has been reduced, and the compressed lophophore has been transformed into a circular one.

Concerning the number of tentacles, Dumortier et van Beneden ('50, p. 46) observe that in the adult there are ordinarily 16, although individuals with 18 tentacles occur not infrequently, an observation which Kraepelin ('87, pp. 98, 99) confirms. In addition to these numbers, I have found 15 and 17. The growth of the odd tentacle is quite interesting. The sections reproduced in Figures 60–62 (Plate VI.) will serve to illustrate a condition which I have quite frequently found in a polypide with 17 tentacles. In this particular series there are only 15 tentacles. The successive sections abundantly demonstrate that the odd tentacle (*) is anal in position, and that it is younger than any

of the others; thus in Figure 61 its tip is cut, in Figure 60 there are only 14 tentacles visible, and these are found in the two following sections. Since there are six sections which pass through the tentacles, and the odd tentacle is found in only three of these, it follows that it is only about one half as long as the others.

The *nervous system* arises, as in Phylactolæmata, by a depression in the floor of the common atrio-pharyngeal cavity, in the region which later becomes the anal surface of the pharynx. As in Phylactolæmata, we first see a shallow pit (Fig. 25, *gn.*). This appears to become deeper, sinking downward and somewhat toward the cardiac valve (Fig. 78, *gn.*). Finally it becomes constricted off from the wall of the œsophagus, and then appears as a cellular mass closely attached to it and surrounded exteriorly only by mesoderm. (Plate I. Fig. 8; Plate VI. Figs. 52, 53, *gn.*) Even before the closure of the ganglionic pocket is completed, the formation of the circumœsophageal nerve, first described by Kraepelin ('87, pp. 62, 63) in the adult, begins.

Figure 52 (Plate VI.) shows a transverse section of a young polypide in which the ganglion is solid, and not provided with a large cavity as in Phylactolæmata. There is a small cavity in the upper part of the ganglion, and this is not yet wholly closed from the œsophagus. The ganglion is continuous with a pair of hornlike processes (*n.*) which partly enclose the œsophagus, and at a later stage do so wholly (Plate IV. Fig. 36, *n'.*) The cells of these horns are found dividing in unusual abundance. The horns lie next to the digestive epithelium, and between it and the mesodermal lining of the ring canal. From the method of growth, and from the sharp line of separation between the tips of the horns and the surrounding tissue, there can be little doubt that the circumœsophageal nerve of Paludicella, like the lophophoric nerves of Phylactolæmata, arises as an outgrowth of the brain.

Serial sections show that the ganglion suddenly diminishes in size immediately below the point at which the circumoral nerves arise, but one can trace a layer of cells continuous with the brain downwards for ten or fifteen micra farther, to near the cardiac valve. At this point one can still see nuclei of a third layer lying between the digestive epithelium of the valve and the mesoderm. It seems to me, therefore, that this may be regarded as a gastric nerve, which seems to originate by a single root and later to give rise to two nerves, one of which lies on either side of the cardiac valve.

7. Origin of the Muscles.

a. Retractor. — After its first formation the bud becomes elongated in the direction of the axis of the branch. The derivation of this elongated stage from the much shorter earlier one might be effected in one of two ways: either, first, by the ingression of cells from the ectoderm at points successively more and more remote from the point of primary invagination, the additions to the length of the bud being made by a continuation backwards of that process by which the first foundations were laid; or secondly, by cell proliferation at the point of first invagination pushing the oral end of the buds farther and farther from the neck of the polypide.

I think there can be little doubt that the second is the method by which the bud becomes elongated; and for the following reasons. (1) The oral end of the bud, on the supposition of continued invagination of the body wall, should become very gradually of less diameter, and transverse sections at that end should exhibit the ingression (potential invagination) of cells which were observed in the earliest stage; but as a matter of fact the oral end is abrupt (Plate III. Fig. 22, 23, *Or.*), and no stages of ingression are to be found there. (2) On the first assumption, the inner layer of the bud should be at all points in equally close relation to the ectoderm of the body wall; on the second, the inner layer should be closely connected with the ectoderm at the neck of the polypide (Plate III. Fig. 22, *cev. pyd.*), but elsewhere it should be sharply separated from it. As a matter of fact, a sharp line can be distinguished, in a sagittal section, separating the inner layer of the bud from the overlying ectoderm at all points except at the neck (anal part) of the polypide (Plate III. Figs. 22–25). Moreover, cross sections of the anal part of the bud show the inner layer passing directly into the ectoderm, and oralward the outer layer of the bud tends to penetrate more and more between the ectoderm and the inner layer. Therefore I conclude that the inner layer of the bud is constantly augmented by cell proliferation in its mass, and especially at the neck of the polypide, and this explanation also accounts for the active cell proliferation observed at the neck in Plate III. Figure 22, *cev. pyd.*

Since the polypide later becomes attached to the body wall by the comparatively narrow "neck" only (Figs. 7, 9, *cev. pyd.*), a separation of the oral part from the body wall has to take place. This process begins at the oral end. In its earliest stages it is indicated by the sharp separation of the inner bud-layer from the overlying ectoderm, and the

partial penetration of the mesoderm on each side into the space between these two layers (Plate IV. Fig. 30, *ms'drm.*). At a later stage the mesoderm may be seen as a single cell layer lying between the ectoderm and the inner layer of the bud midway between the oral and anal ends (Plate IV. Fig. 32, *ms'drm.*), and as a double cell layer at the oral end of the bud (Fig. 34, *ms'drm.*). It is from these cells at the oral end of the bud that the retractor muscles are to arise (Plate III. Figs. 23–25, *cl. mu. ret.*). As the oral end of the kamptoderm and œsophagus to which their inner ends are attached moves away from the ectoderm, and as the area of the latter itself increases, the two ends of the cells move farther and farther apart, and the young muscle cells become drawn out into spindle-shaped muscle fibres. (Plate III. Fig. 25, *cl. mu. ret.*; Plate IV. Fig. 36, *mu. ret.*) The retractor thus arises unpaired and remains so at its origin, but nearer its insertion in the ring canal and œsophagus one can distinguish a division into right and left masses. The adult muscle fibres consist of two parts at least, the inner contractile portion and an outer less modified protoplasmic portion, which can be traced over the whole of the first part, but is most evident around the nucleus, where it has a granular appearance.

b. Pyramidalis. — At about the stage of Figure 25 (Plate III.) one finds, on cross sections of the branch which pass through the neck of the polypide, that the mesoderm of the body wall on each side of the neck is greatly thickened, and that its closely packed cells, which lie three or four deep, have become somewhat elongated. Cell division is quite common in the ectoderm of this region, and by it the area of the circumcervical region is increased and the two ends of the muscle fibres are carried farther apart, one end remaining attached to the neck of the polypide and the other moving towards the abatrial surface. I have given reasons above (page 16) for believing that the abatrial ends of the muscles are not carried towards the abatrial side passively, and solely by the growth of the body wall, but that the ends move relatively to the cells of the body wall. A somewhat late stage in the development of the pyramidalis is shown in Figure 63 (Plate VI.). Nearly the whole of the mesoderm of the body wall has here been transformed into muscle cells. The insertion of the muscles is in the mesoderm of the neck of the polypide. (Plate VI. Fig. 63; Plate V. Fig. 45.)

c. Parietal muscles first make their appearance at about the stage of the terminal individual of Plate II. Figure 14, immediately below the bud and to the right and left, i. e. so that the muscles, which usually arise paired, have their long axes parallel to the sagittal plane

and perpendicular to the long axis of the branch. They arise from cells of the mesoderm, most of which in this region are filled with vacuoles, and often project into the cœlom. But in my opinion the muscle cells do not themselves arise from such vacuolated cells, for at even an earlier stage (corresponding to Figure 21, Plate III.) one can distinguish thickened patches of elongated cells in the mesoderm which are undoubtedly the young muscle cells; but they do not show the slightest traces of being vacuolated, and in fact are sharply distinguished from the adjacent cells by their uniformly granular appearance and their deeper coloration.

Braem ('90, pp. 124, 125) has already stated that the parietal muscles arise in pairs, and come to traverse the cœlom, not remaining in the body wall. The truth of this statement I can confirm in the case of the parietal muscles first formed, which lie near the future septum. Plate V. Fig. 42 shows the origin of the muscle fibres on both sides of the branch. They have already migrated into the cœlom. As Braem plainly states, the component parts of this pair of muscles, developed from the mesoderm, migrate towards each other and finally fuse into one unpaired mass, as we see in Plate III. Figure 26. It is perfectly evident, in this case at least, that both ends of two muscles originating far apart migrate in some manner towards each other so that the corresponding ends come to lie close together. Such a migration cannot be accounted for merely by growth of the body wall. The ends of the muscle fibres must move relatively to the body wall.

When the muscles have reached their permanent positions in a diameter of the branch, we find their ends attached to the cuticula. As the muscle fibres stain deeply in hæmatoxylin, they can be distinctly traced through the vacuolated and poorly stained cells of the body wall (Plate III. Fig. 26). Figure 29 shows a bit of the wall mechanically separated from the cuticula, the end of the muscle fibre remaining in place. Fine lines can be distinguished in the contractile, deeply staining portion of the fibre. The surface by which attachment is effected appears very slightly crenulated on longitudinal sections of the muscle fibre. I could not distinguish any structural peculiarity on the part of the cuticula to which the muscle was attached, — nothing to indicate how attachment is effected.

Freese ('88, pp. 15, 22, Fig. 11) has described a similar method of attachment of the muscles to the cuticula for Membranipora.[1]

[1] My friend, Dr. G. H. Parker, tells me that a similar method of attachment of muscle fibres to the cuticula occurs in *Crustacea*. According to Tullberg ('82, pp. 27, 44, 45), the adductor muscle fibres are in Mollusks attached to the cells of the ectoderm. The same condition as in Mollusks seems to exist in Annelids (Eisig, '87, pp. 25, 36)

At a later stage smaller bundles of muscles arise successively toward the neck. These muscles are free from the body wall at their middle region. They do not usually pass through the cœlom in a diameter of the branch, however, but rarely subtend as chords an arc of more than 120°. As Braem supposed, such muscles, although arising later than the most proximal pair, originate in a similar manner to them (Plate VI. Fig. 55). The mesoderm is very thin at the region at which they are first seen, and they are quickly discerned by their larger nuclei and prominent cell body. At a later stage they have grown much longer, and become freed from the body wall at their middle part.

As is well known, there are two *funiculi* in Paludicella, called by Allman respectively anterior (nearer the atrial opening) and posterior. The origin of the funiculi of Paludicella was observed by Dumortier et van Beneden as long ago as 1850. They say (p. 54), "La couche muqueuse une fois formée s'étend rapidement dans l'intérieur et touche bientôt par son extrémité inférieure les parois opposées de la loge. Les cellules muqueuses dont le tout est encore composé contractent de l'adhérence dans cet endroit, et c'est ce qui donne naissance au muscle rétracteur de l'estomac [= funiculi]." Allman ('56, p. 36, Plate XI. Figs. 7–9) also describes and figures very clearly and correctly this process, and Braem ('90, p. 127) has recently confirmed their observations.

It is perhaps unnecessary to redescribe the more evident part of this process, the contact of the polypide with the abatrial wall of the branch. The mesoderm of the bud comes into contact with that of the body wall, the cells of each of the two layers become attached to the other, and by the withdrawal of the polypide the attachment persists at two points forming a long drawn out string of tissue. Figures 36[a] and 38 (Plate IV.) are contributions to a knowledge of the finer details of this process. Apparently the upper funiculus is developed earlier than the lower, as I have always found it longer at about this stage. The lower funiculus at present consists of only the two mesodermal layers of body wall and polypide intimately united. The funiculus itself consists of a cord several cells thick; but I believe these certainly to be derived from the mesoderm only. Very early some of these cells show an appearance of highly refractive and deeply staining fibres, which I interpret as muscular differentiation (Plate IV. Fig. 38, *fun. su.*), so that the funiculi must be regarded as partly muscular in function. As in Phylactolæmata, these fibres lie near the axis of the funiculus. Braem ('90, pp. 66, 67,) has demonstrated that the muscular fibres of the funiculus of Plumatella pass directly into the muscularis

of the body wall. It is interesting to find them persisting in the funiculus of Paludicella, beneath the mesodermal covering, although there is apparently no muscularis developed in the body wall of this region.

8. The Formation of the Neck and Atrial Opening.

This is the last act in the history of the polypide that I shall consider. The body wall around the neck of the polypide continues to possess a less differentiated character than the remaining portion for some time after the oral tentacles have undergone their revolution. One still sees the cells of this region dividing, and the body wall is gradually protruded at this point above the general level. (Plate II. Fig. 14, *cev. pyd.*) The neck of the polypide to which the kamptoderm is attached consists, at a somewhat earlier stage than that just referred to, of a disk of greatly elongated columnar cells in the centre of which there is a distinct notch caused by the presence of shorter cells at that point. (Plate VI. Fig. 63 *b*.) At the inner ends of the columnar cells of the neck lies a flat epithelium quite sharply marked off from the latter, but which is nevertheless undoubtedly derived from the same source as the columnar cells and the inner layer of the bud. This flat layer is directly continuous with the inner layer of the kamptoderm. At a later stage, the columnar cells of the ectoderm become elongated still more, and lose their staining capabilities at their outer ends. Still later one sees them arranged in the form of a cup whose cavity is separated from the outside world only by a cuticula which becomes slightly invaginated at this point. The cells are soon found with their long axes perpendicular to the edge of the cavity they line.

There is one point that I have not been able to determine; namely, how the new cuticula, which is certainly formed at the ends of the cells which lie next to the cavity, becomes continuous with the old cuticula of the non-invaginated body wall, as it is in Figure 50 (Plate V.). The presence on the new unstainable cuticula of the remains of the stainable one, whose origin I have already discussed at length, may serve as a guide to the limits of the old cuticula. The new cuticula is being secreted by cells lying deep in the inner end of the neck, and apparently in one rod-like mass. Unfortunately, I lack stages between this figure and Figure 45 (Plate V.), which shows the neck of a nearly or quite adult polypide cut lengthwise. The solid cuticular rod has now become a hollow cylinder, whose inner (deep) edge is embedded in the deep-lying cells of the neck. Moreover, one finds superficial to the cuticula of the general body wall a second cuticular cylinder, which is free at its outer end,

but at its inner end fuses with the surrounding cylinder of cuticula. This inner cylinder, which is probably formed, as Kraepelin ('87, p. 40) suggested, by splitting of the delicate cuticula at the base of the marginal thickening (Randwulst), has been compared by Kraepelin to the "collare setosum" of Ctenostomes. The *Randwulst* itself I believe to be the equivalent of the Diaphragma of Nitsche, as I shall try to show later.

At the deep end of the neck (Fig. 45), the inner layer of the bud is seen to be continuous with the ectoderm. The region of transition may be called the atrial opening, *of. atr.* Surrounding the atrial opening is a fold in the ectoderm, and between the layers of this fold is a thin, non-stainable homogeneous layer, slightly more refractive than the surrounding protoplasm. This membrane extends also a short way into the kamptoderm, and here lies between its two cell layers. Embedded in this homogeneous membrane in the fold, one can distinguish still more highly refractive bodies, *spht.* On account of their form and high refractivity, I believe these to be muscle fibres cut across. The homogeneous membrane has also the same general appearance and relation to the muscularis as the so-called supporting membrane of Nitsche, and it is the only representative of that structure that I have found in Paludicella.

9. Development of the Communication Plate.

In their description of Paludicella, Dumortier et van Beneden ('50, p. 40) say : "Il se compose de plusieurs loges ou cellules placées bout à bout . . . en sorte qu'il n'y a aucune communication entre les différents animaux." Also Allman ('56, pp. 114, 115) refers to the presence of a perfectly formed septum separating the cavities of adjacent "cells." To Kraepelin ('87, p. 38) belongs the credit of having first carefully studied this structure in the adult by means of sections. He came to the conclusion from the appearances which he figures (cf. my Plate V. Fig. 49), that there are small canals passing through the nearly homogeneous central mass, and therefore "dass wir in dem ganzen Apparat eine Vorrichtung zu erblicken haben, durch welche Nährstofflösungen des einen Tieres mittels siebartig wirkender Cautelen in die Körperhöhle des Nachbarindividuums übergeführt werden."

The descriptions of Kraepelin concerning the structure of the "Rosettenplate" are confirmed by my own observations, and seem to justify his conclusions concerning its function. The development of the organ has not, however, been carefully observed heretofore. Korotneff ('74, Plate XII. Figs. 1 and 2) gives figures to show this process, but I have never

seen any such circular groove surrounding the branch as he figures. In all cases the two layers of the body wall form a circular fold, in which, however, there is never, even at the earliest stages, a space between the ectodermal layers, nor any infolding of the cuticula as Korotneff ('75, p. 369), according to Hoyer's rather incomplete abstract, maintains (Plate V. Fig. 47). When the circular fold has advanced until only a small pore remains, by which the cavities of the older and younger individuals are kept in communication, the mesodermal cells at the angle of the fold begin to undergo a metamorphosis both in form and histological character. In the first place they become much elongated and extremely attenuated, passing from one surface of the septum to the other, and forming the lips of the pore. In the second place their plasma becomes first deeply stainable, and later, in addition, homogeneous and highly refractive. These metamorphosed cells form what may be called the *teeth* of the plate. They are derived wholly from mesoderm.

The cells in the upper mesodermal layer next increase rapidly in number and size, and the number of teeth is also augmented (Plate V. Fig. 48). The metamorphosis of the cells extends still farther away from the communication pore, and involves the lower mesodermal layer; but, apparently, each cell of the latter is metamorphosed only to a slight depth within its cell wall (Fig. 51), whereas in each of the upper cells the ends which project into the communication pore are modified through and through (Fig. 46). At a later stage (Fig. 49) the metamorphosed part of the cell seems quite sharply cut off from the active part, and the slits between the metamorphosed teeth are considerably reduced. Nevertheless, I believe a transfer of fluids may still occur between them, for even in the adult communication plate one can trace continuous lumina when the cells are by accident torn off from the "teeth" which they have produced. It is important to note that the nuclei are not destroyed in the cell metamorphosis. Some lie above, others below the pore, and become deeply stainable. The ectodermal layers of the communication plate secrete a cuticula between them. This is thinner than that of the body wall, and does not extend, of course, to the centre of the communication plate, but ends in a thickened ring, whose diameter is about one tenth the diameter of the plate, or, absolutely, about 9.4 μ.[1]

[1] Reichert ('70, p. 267) first carefully described the Rosettenplate of Ctenostomes in Zoöbotryon, and the organ in Paludicella must be regarded as homologous with it. The central circular hole in the cuticula of Zoöbotryon is from 7 to 10 μ in diameter, and from one ninth to one seventh that of the entire plate. Similar

10. Rôle of the Mesodermal Vacuolated Cells.

Allman ('56, p. 36) observed that at the time a lateral branch was well formed, and before the origin of the polypide, the internal outline of the body wall was uneven, and he figures (Plate XI. Fig. 4) very large cells lying on the inside of the body wall. Korotneff ('74, Taf. XII. Figs. 1–3, '75, pp. 369, 370) progressed a step farther, and recognized a distinction between large, coarsely granular cells projecting into the cavity of the bud, especially near the tip, and the surrounding epithelial cells. Braem ('90, p. 126), finally, has described them more accurately. He finds cells filled with numerous granules in the youngest branches of the colony. Immediately around the bud, such cells are less abundant; probably, he says, because their granules have been absorbed in the process of formation of the polypide. He compares the granules with the yolk spherules of the statoblast cells, and believes that they are to be regarded as food matter.

My observations and conclusions, achieved independently of Braem's, fully confirm his. I have succeeded, moreover, in obtaining some additional evidence as to the function of these cells, a subject to which I have paid some attention.

First as to the *distribution* of the cells, and their frequency in different regions. We can best get an approximate idea of this by counting the number of the reticulated cells in each section of a series which involves a young polypide and the regions immediately above and below it. It is not possible to do this with perfect accuracy, because there is no sharp line of distinction between reticulated and non-reticulated cells; but I have made the count without prejudice, and I believe as fairly as possible. When the bud of the polypide has reached about the stage shown in Plate III. Figure 28, the number of reticulated cells seems to have nearly reached a maximum. In the series from which this figure was taken there was an average of 4.8 reticulated cells to the section in the ten sections distal of the bud. There was an average of 11.2 reticulated cells to the section for the twenty sections which passed through the bud, and 11.2 for the eleven sections proximal of the bud in the region

perforated organs have been described by Smitt ('67, p. 426), Nitsche ('71, pp. 420–422), and Vigelius ('84, p. 26) for Flustra, by Freese ('88, p 7, 13, 14) for Membranipora, by Ostroumoff ('86[a], p. 13) for Lepralia, by Claperède ('70, p. 160) for Bugula and Scrupocellaria, by Ehlers ('76, p. 14) for Hypophorella, and by Joliet ('77, p. 222) for Bowerbankia. Nitsche alone ('71, p. 455) has had anything to say upon their origin, and this apparently not the result of direct observation.

at which muscle fibres were arising. A similar series through a slightly older bud gives for the same regions respectively 5, 14, and 13 cells per section. In series through older buds, a rapid decline in the number of these cells occurs so that at the stage of Figure 30 (Plate IV.) there is an average of only about 3.1 cells per section through the bud, and about 2.2 immediately below. These reticulated cells are not very numerous in the region of the bud at the time this is about to arise, as a look at the sections Figures 3 and 4 shows. One finds reticulated cells in the mesoderm at the tip, and most abundantly at a rather early stage in the development of the bud. The number of these cells diminishes as one leaves the young individual to pass into the next older of the same branch. In the adult such cells are rather rare; so rare, in fact, that Kraepelin ('87), who studied with care the body wall of the adult individual, makes no mention of them. Nevertheless they do occur in the cells which are to go into the lateral branch (Plate II. Fig. 15), as well as elsewhere on the body wall. The place in which one finds the reticulated cells most abundant, however, is in the young *lateral* branches near the time when the polypide bud is about to arise. Here every cell of the mesoderm is greatly enlarged, and filled with the vacuoles (Plate VI. Fig. 58). These are very apparent upon a surface view of the branches. Reticulated cells occur not only in the mesodermic cells of the body wall, but also in those of the polypide bud, which were, indeed, only lately a part of the mural mesoderm (Plate III. Fig. 28, Plate VI. Fig. 56). Thus, in general terms, we may say that the reticulated cells of the mesoderm are chiefly confined to regions in which there are young buds developing; and since these arise at intervals only, there is a periodicity in their appearance, — a time of maximum development followed by one of decline, then one of reproduction of such cells in the ends of branches culminating in another maximum, and so on.

Turning our attention now more particularly to the *structure* of these reticulated cells at the period of their best development, we find (Plate VI. Figs. 56, 57, 59) that they possess a large nucleus lying at the deep end of the cell and containing a relatively large nucleolus, and that this is surrounded by a granular protoplasm with included vacuoles. It is very common to find the nuclei in various stages of division, and thus it is frequently seen as a mass of chromatic substance without any nuclear membrane or nucleochylema. The vacuoles, which in the more regular cells lie in a semicircle nearly peripheral (the nucleus being at the centre), are highly variable in number, some of the cells containing as many as 20 to 30. They often appear as perfectly clear homogeneous

spaces, but more frequently at this stage contain a spherical body, which frequently fills the entire vacuole and is more refractive than the surrounding plasma (Fig. 59). Not unfrequently one sees a less refractive, clear space, surrounding the highly refractive body (Fig. 57).

The description just given corresponds to the condition seen in a terminal branch whose polypide has attained the development of that shown in Figure 28 (Plate III.). At the time immediately preceding the origin of the bud, the cuboidal cells of the mesoderm show traces of vacuolation, but their form and size have suffered no appreciable disturbance. This vacuolation of cells proceeds hand in hand with the development of the bud, and one first notices the homogeneous, highly refractive bodies in the vacuoles when the bud is well established. At about the time the alimentary tract has become formed, the reticulated cells begin to show signs of degeneration. The highly refractive bodies have disappeared, and the skeleton of the cell which remains becomes very irregular. As already stated, the number of reticulated cells also decreases, until, at about the time of "rotation" of the polypide, there are few reticulated cells in the mesoderm, but these few are filled with vacuoles and their highly refractive bodies.

The conditions of the mesodermal cells at the tip are slightly different from those found elsewhere. Usually, instead of many small vacuoles, one finds only one or two which fill almost the entire cell, — sometimes perfectly homogeneous in structure, sometimes containing small highly refractive granules.

These appearances I believe to be explicable only upon the assumption that *the mesodermal cells are capable, at the time at which the young polypide is arising, of imbibing the fluids of the body cavity and storing them up for the purpose of supplying the rapidly growing cells of the bud with nutrition.* It is desirable to show reasons for believing, first, that the contents of these cells are nutritive matter; secondly, that this has been taken up from the body cavity; and, thirdly, that it is supplied to the bud for its nutrition.

It must be admitted that the strongest argument for the belief that these are absorbing cells is derived from a comparison of the appearances which we find in these cells with those described for Protozoa, and by Metschnikoff ('83, Taf. I. Figs. 18–35) for mesodermal trophic cells. At the same time, it must be acknowledged that similar cells are found in other cases where the function is believed to be not ingestive, but excretory, as in the chlorogogen cells of Annelids, as shown by Kükenthal ('85), Eisig ('87, pp. 751–762), and others, and indeed even in the

cells of cœlomic epithelium. Eisig ('87, p. 752) has already clearly expressed how, in view of the many cases of high excretory activity of peritoneal and blood cells demonstrated by him, "künftighin bei der Beurtheilung gewisser Zelleneinschlüsse erst genau festzustellen sein wird, ob man est mit *von aussen aufgenommenen* (gefressenen), oder aber mit von der Zelle *ausgeschiedenen* Producten zu thun habe."

A criterion for judging this matter may be found, in the first place, I believe, in this: that the products of excretion increase with the activities of the cells, and are thrown out, usually in the shape of concrements, either *from* the cell or *with* the cell into the cœlom; whereas bodies taken in from without for digestion decrease with the activities of the region. In the second place, vacuoles are less characteristic of excretory tissue than of imbibitory. But vacuoles are the important feature of the reticulated cells in Paludicella, and the highly refractive bodies are less constant phenomena. As for the latter, they are not found in the later stages, nor in the earliest. Moreover, these bodies differ from excretion concrements in this, that they are always transparent, often almost indiscernible in the vacuole, except by their higher refractiveness, and there is no sharp demarcation between cases of vacuoles filled by such bodies and those the contents of which are less highly refractive. The degree of refractiveness is variable, at one end of the series grading off into the undifferentiated fluid of the vacuole. What significance is to be assigned to these highly refractive bodies in the vacuoles? There are two reasons why I do not believe that they represent solid food particles devoured as such by the mesodermal cells. First, I do not find such highly refractive bodies lying loose in the body cavity before the stage at which they first appear in the cells; and, secondly, one can find all gradations between less highly refractive vacuoles and highly refractive ones (which I have assumed to be entirely filled by one highly refractive body), and between the latter and vacuoles containing a small body surrounded by a broad, clear area. I believe, therefore, that the vacuoles are rather cavities filled with chemically different nutritive fluids, which are acted upon differently by the reagent.

I have assumed that the contents of the vacuoles represent material taken up from the body cavity, because it seemed most reasonable to look there for the source of their supply. The ectoderm is covered on its outer surface by an apparently continuous cuticula, so that food cannot be gained from the outside world directly. It is, moreover, not unreasonable to suppose that some of the products of digestion elaborated by the adult polypides of the colony pass through the wall of the

alimentary tract in solution, and thus into the body cavity, from which they may be taken up by the mesodermal cells at the growing part of the body wall. Nor is there anything unreasonable in insisting that the body cavity functions, in these animals without blood-vessels, as a hæmo-lymph system, for in many animals with incomplete vessels, such as Arthropods, Hirudinea, etc., it evidently does so to a certain degree. Moreover the constant motion of the fluids of the body cavity of Bryozoa points to the same thing. It is conceivable that the food in the digestive cells might be distributed throughout the body wall without passing into the body cavity, since all parts of the body wall are continuous with the digestive epithelia of the polypides of the colony. Two considerations make it improbable that the cells of the tip gain their nutrition in this manner from the digestive cells of the youngest functional polypide: first, the considerable distance of the rapidly growing, and hence rapidly consuming tip, from the youngest functional polypide; and, secondly, the fact that the tip is separated from that polypide by one or two septæ, whose central cells are highly metamorphosed, and apparently cuticularized, thus serving to break the continuity of the cell wall. An objection to the assumption that the mesodermal cells of the tip derive their nourishment from the products of digestion which have been elaborated by the alimentary tract of the youngest polypides and passed into the body cavity, might be based on the fact that the communication plates are always fully formed between the bud and the next older polypide before the older polypide has become functional. If the communication plate were a closed septum, this would be a fatal objection. But it is not closed to fluids carrying food in solution. The very persistence of an opening indicates that it has a function, and favors the hypothesis here presented.

Positive evidence for the conclusion that the reticulated mesodermal cells take up food material from the body cavity is derived from the fact that these cells often show evidences of being amœboid. Thus they are sometimes found with pseudopodia-like prolongations of the cell body (Figs. 54 and 59). A large percentage of all reticulated cells of this stage show similar appearances. Although they here seem to keep their places in the mesodermal epithelium, their movements being confined to their free surfaces, the cells derived from the homologous layer in marine Bryozoa are migratory. Therefore these may be considered as morphological equivalents of migratory cells, which have come to remain in or have never departed from the mesodermal layer, although possessing some of the characters of these notoriously trophic elements.

That the nutritive matter in the cœlomic cells is supplied to the young bud is what we should expect, since the cells of the bud, being most actively engaged in growth, will require most nutriment. The actively dividing cells of the outer layer of the bud are thick and cuboid, and are rarely so highly vacuolated as the more passive ones of the body wall; yet occasionally one finds one or two huge cells in this layer full of vacuoles, which contain highly refractive bodies. In most cases these cells send out processes into the cœlom, and in a few instances I have seen them united with similar processes from cells on distant parts of the body wall. This remarkable phenomenon, shown in Figure 54 (Plate VI.), may possibly signify that cells of the cœlomic epithelium at times directly communicate with those of the outer layer of the bud to supply it with nourishment. Nutrition of the bud is also probably effected through the presence of large reticulated cells at the angle between the bud and the body wall. A condition like that shown in Figure 56, *cl. ret.*, is very common.

Every author from Dumortier et van Beneden to Braem, who has studied the origin of the polypide in Paludicella, has mentioned the presence of highly refractive bodies in the alimentary tract at the time of its formation. These are very striking in *some* living specimens, and in whole animals after killing. I have found that this highly refractive substance in the bud is exceedingly variable in amount and position, and that sometimes it is apparently absent. When present, it usually occupies the lumen of the forming alimentary canal; but, as sections show, it is often located in large vacuoles in the future digestive cells of the alimentary tract. It seems highly probable that, as Braem suggests, this is nutritive substance, and it has doubtless come from the body cavity through the agency not only of the outer layer of the bud, but also of other parts of the cœlomic epithelium.

I am inclined to interpret the phenomenon of cells filled with nutritive material as an adaptation to the peculiar conditions of Paludicella, in which the individuals are early separated from one another, except for the communication plate, through which at best fluids can pass only slowly, and in which a rapid growth of the body wall to produce the polypide takes place periodically. The mesodermal cells rapidly absorb the nutritive fluids of the body cavity and store them in their substance before the formation of the communication plate, and give them out again during the period of the polypide's most rapid growth chiefly to this part of the individual. This hypothesis has been mainly derived from considering the fact of the great development of the reticulated

cells in the lateral bud and the very early completion of its communication plate, the immediate needs of the polypide, which arises only after the formation of the plate, being met by this supply of stored nutriment.[1]

But why is the septum (communication plate) formed so early, if it is desirable for the species that the growing tip should be well nourished by the fluids of the body cavity? Here again I must resort to pure hypothesis. I assume that the early formation of the septum is a provision for the protection of the stock against a rapid influx of the surrounding water in case the branch is broken. One can understand how, if the body wall and growing regions depend upon the fluids of the body cavity for nutrition, an open communication of this cavity with the outside world would be a serious obstacle to regeneration of the body wall in the lost part, or the growth of the stock in any other direction. There is a fact which ought to be mentioned in this connection, as bearing on this hypothesis of the function of the septæ. One frequently finds that in stocks which have been handled with reasonable care the median branches are broken off at either end, and in almost every colony one or more lateral branches are missing from the parent branch. Apparently, then, the lateral branches are unusually subject to destruction, and we find the septæ developed at a much earlier period between them and the ancestral branch than between individuals of the median branch. Compare Plate II. Figure 14, in which the communication plate has not yet begun to form, with Plate VI. Figure 58.

III. Budding in Marine Gymnolæmata.

1. Architecture of the Stock.

I have already described the process of stock-building in Paludicella, and have attempted to show that it follows a certain law. I desire now to present a few observations upon the architecture of certain stocks of marine Gymnolæmata, which will aid in arriving at some general conclusions later on. Other observers have worked out the architectural laws of single species or groups, and I shall refer to their studies either

[1] Similar conditions to those in Paludicella exist in some marine Bryozoa, and in one of these cases, Bowerbankia, I find them fulfilled by a similar arrangement. The young buds of the stolon which give rise to the "nutritive zoöids" are, at an early stage, loaded with food granules. As in Paludicella, so in Bowerbankia the communication plates are formed early.

in connection with the species which I have used in common with them, or in the general part of this paper, in considering the process of budding in Bryozoa as a whole.

I will begin my description with *Bugula turrita*[1] of Verrill, which I gathered in the summer of 1889 at Wood's Holl, where it occurs abundantly on the piles of the wharf. The stock is bushy, and, when its polypides are active, of an orange color. In its simplest form the stock consists of a central axis, which is somewhat zigzag, and gives off lateral branches like the trunk of a tree. The lateral branches are inserted on the trunk in a spiral line. Each lateral branch is fan-shaped (Plate VII. Fig. 64), the part corresponding to the handle of the fan being the point of attachment, and the fans are smaller the nearer they are to the tip of the trunk. The attachment of the branch to the trunk is effected by one primary individual. Each fan-shaped branch extends from its point of attachment obliquely upward and outward, and, although it is slightly concave on its upper inner surface, the concavity is not sufficient to prevent its being spread out upon the slide for study without materially disturbing the interrelation of the individuals in the stock.

I have studied several branches flattened in this way (one of 400 individuals), and have made camera drawings of them. Since the results in the different cases are substantially in agreement, I have concluded that they are significant. One of these camera drawings is shown in the figure just referred to.

To designate individuals in the stock, I have adopted a simple nomenclature. The forty-four terminal individuals are numbered from 1 to 44. The successive generations (if I may be allowed to use this word in a loose way) are indicated by the Roman numerals from I. to XIII. Any one individual is indicated by placing the numbers of the radial line or lines to which it belongs first, and following this by the Roman numeral of the generation to which it belongs. Thus, 27–30 IV. is an individual near the base of the twig 27–30 and of generation IV. Figure 64[a] (Plate VII.) is a diagram showing the

[1] This species is very similar in general habit to B. avicularia, Linnæus, and to B. turbinata, Alder (Hincks, '80, pp. 75–80). It differs from the first named species by possessing only one spine, on the outer upper edge, as described by Leidy ('55, p. 142), instead of having three, — two outer upper and one inner and upper. It differs from Hincks's diagnosis of the second in having only two "cells" in each branch, instead of 3–6 in the upper portions. The form of the avicularium would seem to ally it more closely to B. avicularia.

arrangement of the individuals in Figure 64. The radial lines represent the rows of individuals; the concentric lines separate adjacent individuals of the same radial row. The same nomenclature is used as in Figure 64.

In studying Figures 64 and 64[a], one of the first facts which attracts our attention is that (1) *the individuals of the twigs are in pairs, and the adjacent individuals of the two rows "break joints."* In general, one finds that the individuals of the same twig are of the same length; but since the two rows of any twig ultimately rest upon one, either the proximal two individuals of these rows must be of unequal length, or else they must arise on different parts of the individual which supports them. Both of these cases occur. Sometimes one individual (26 IX.) has nearly twice the length of the other (25 IX.), and in other cases (9, 10 VI., 11, 12 VI.) the more proximal of the two individuals (9, 10 VI.) arises so far proximally on the side of the supporting individual 9–12 V. as to have a total length quite equal to that of the more distal (11, 12 VI.). Owing to their different positions upon the individual 9–12 V., these two individuals may be designated as lateral (9, 10 VI.) and terminal (11, 22, VI.). *The terminal individuals continue the ancestral row; the lateral individuals are the first of lateral branches.*

This distinction is an actual, and by no means a meaningless one. The constant difference in position of the two individuals which rest upon one shows conclusively that this branching cannot be regarded as dichotomous, and I may say parenthetically that I shall try to show in the general part of this paper that true dichotomy is not common in Bryozoan stocks, if indeed it exist at all. Now, since in the rows of individuals in which there is no lateral budding the distal lies directly terminal to the proximal individual, that individual which fulfils this condition at the region of bifurcation of the twig must be regarded as continuing the ancestral branch; and that individual, conversely, which arises from the side of the single proximal individual must be regarded as the lateral one. Thus we have the stock composed of ancestral and lateral branches as represented in Figure 64[a].

(2) *When two lateral branches are given off from two ancestral ones which have had a common origin* (and are consequently themselves respectively ancestral and lateral branches), *they are given off towards each other.* This is equally true whether the two lateral branches in question arise in the same generation (32 X., 33 X.) or in different

generations (24 X., 25 IX.). This may be expressed by saying branches are given off on the side towards the axils.

By consulting Figure 64[a] and tracing out the finely dotted lines which connect the second, third, etc. axils of all branches counting from the proximal end of the fan, it will be seen that (3) *lateral buds tend to arise on two closely related branches in the same generation.* There are several slight deviations from this rule. The less closely related the branches, the less marked the tendency, although it is still discernible. (Cf. branches 9–16, 23–30.)

This rule does not hold, however, so well on the margins as in the middle region of the fan, for here another and a superior rule seems to obtain. This is that (4) *lateral budding occurs more frequently at the margins of "fans" than elsewhere.* Thus in Figure 64[a] there is at the margins, on the average, 1 case of lateral budding to 4.3 cases of median budding. Elsewhere the average is as 1 to 6.5. In larger fans the difference is even more pronounced. This is true not only for the "fans," but also, to a less degree, for the two "subfans" which arise respectively from the two individuals of generation II. (but 17, 18 is very anomalous in this respect). In general, any rule deduced for the margin of the fans holds true also for subfans to any degree of subdivision; but the less perfectly, the higher the degree.

By consulting again the diagram, it will be seen that the branches have attained different lengths. Thus 9, 10, 29, and 30 contain representatives of generation XIII., while the terminal individual of branch 1 is of generation X., and those of branches 35–44 are of generation XI. So the curve which connects the tips of the branches (see dot-and-dash line, Fig. 64[a]) would rise from 1 to 9–10 as a maximum, and fall again till it reached the margin of the first subfan; then rise again, reaching a second maximum in the middle at 29–30, and finally fall again to the other margin. In general, then, (5) *the marginal branches are shortest, the intermediate ones longest,* i. e. *give rise to the greatest number of generations.*

Although the marginal individuals of say generation III., IV., or V. do not support branches with so many generations as the intermediate ones, yet they are not therefore necessarily less prolific in individuals, because the number of branches arising distally of such individuals is greater according to rule 4 than the number arising distally of the intermediate ones. Thus, if we count the number of individuals borne on each of the eight individuals of the fourth (IV.) generation of Figure 64, we find in the given case: —

Outer.	1.	1–8 IV.	(an outer	individ.)	gives rise to	8	branches	and	34	individ.
	2.	9–12 IV.	(an inner	")	" "	4	"	"	27	"
Inner.	3.	13–16 IV.	(an inner	")	" "	4	"	"	23	"
	4.	17, 18 IV.	(a subouter	")	" "	2	"	"	13	"
Inner.	5.	19–22 IV.	(a subouter	")	" "	4	"	"	25	"
	6.	23–26 IV.	(an inner	")	" "	4	"	"	23	"
Outer.	7.	27–30 IV.	(an inner	")	" "	4	"	"	26	"
	8.	31–44 IV.	(an outer	")	" "	14	"	"	56	"

According to the rule that inner branches are slightly prolific, we should expect cases numbered 4 and 5 in the above table to contain the fewest branches and individuals; in accordance with the rule that marginal branches even of subfans are more prolific, we should expect them, on the contrary, to contain more branches and individuals than cases numbered 3, 6, etc. The result is usually a condition intermediate between that of the middle and outer branches, such as is partially realized in case number 5. Case number 4 seems to present an unusual condition, which may be correlated with the fact of its close approximation to number 5. (See Fig. 64, 17–20.) From the consideration of this and other cases, I think this conclusion may fairly be drawn: (6) *Of the four proximal individuals from which a fan arises, the outer two will bear the greater number of individuals, the inner two the lesser.*

Since from rule 2 median individuals (ancestral branches) occupy the margins of fans (or subfans of any degree) and the lateral branches are intermediate, it follows, as a corollary to rule 5, that, in general, the ancestral branches are the shorter, the lateral branches the longer; and, as a corollary to rule 6, that from any axil the ancestral branch will of the two give rise to the greater number of individuals; the lateral branch, conversely, to the less, other conditions being equal.

We have deduced the laws of lateral budding on different parts of the circumference. We find also that there is a regular variation in the frequency of lateral budding, dependent upon the distance of the region from the primary individual of the fan. This rule, like any other, is not invariable, whatever the other conditions may be; but it is more or less dependent upon them. A small and regular fan having seven generations gives this result.

No. of Generation.	Number of Individ.	Increase per Cent.
I.	1	
II.	2	100
III.	4	100
IV.	8	100
V.	12	50
VI.	16	33⅓
VII.	16	0

In this table the first column gives the number of the generation, the second the whole number of individuals in the generation, and the third column the increase per cent of individuals in each succeeding generation over the last. In this specimen the increase underwent a very regular diminution.

With larger colonies so great a regularity as that just shown is hardly to be expected, nor is it found. The following table is based on Figure 64, and is like the preceding; but in addition the percentage increases have been averaged — i. e. the means of successive increases taken in pairs have been given — to eliminate what may be called accidental variations.

Generation.	Number of Individ.	Increase per Cent.	Average.	Generation.	Number of Individ.	Increase per Cent.	Average.
I.	1			VIII.	28	27	29
II.	2	100	100	IX.	33	18	23
III.	4	100	100	X.	39	18	18
IV.	8	100	88	XI.	44	13	16
V.	14	75	49	XII.	20	Incomplete.	
VI.	17	22	26	XIII.	4		
VII.	22	30					

Hence we conclude, *There is a diminution in the rate of increase of individuals in the "fan" as it grows older.*

In searching for an explanation of this phenomenon, I first drew a line from the centre of the primary individual of the fan to the periphery, and divided it into four equal parts. I then described arcs with the primary individual as a centre, and with radii equal to $\frac{1}{4}$, $\frac{2}{4}$, $\frac{3}{4}$, and $\frac{4}{4}$ of this line respectively. Counting the number of individuals cut by these arcs respectively, and dividing those numbers by the length of the corresponding arcs, I found that *there is almost exactly the same number of individuals per unit of arc for each of the four arcs.* (Rule 7.) The previous conclusion, that there is a diminution in the rate of increase of individuals in the fan as it grows older, may then be considered as a corollary to this rule, as it obviously follows from it.

Bugula flabellata, J. V. Thompson.[1] — I have studied this species for the purpose of confirming the results obtained in B. turrita, and have found the architecture of the two species alike in all essentials.

The entire colony of B. flabellata (Plate VII. Fig. 66) may be compared to a single "fan" of B. turrita, only there are usually many more individuals in the former, and of course there is no central stem to which it is attached; but the fan is fastened directly by its rhizoids to the object which supports it.

Usually about four rows of individuals are united, instead of two as in B. turrita, — a condition which can be easily derived from the latter by imagining adjacent branches to become fused together. Here as there adjacent individuals break joints. Here as there lateral branches are given off towards the axils.

Rule 3 is not true for B. flabellata. This is entirely annulled by the establishment of a new rule, which depends upon the new conditions found in this species; namely, that more than two rows cling together, and that consequently one or more rows of individuals are enclosed between outer marginal rows. In any such twig composed of more than two rows (Rule 3a) *lateral branches are given off only from the marginal rows.* (See Fig. 66, 49–54 XVII.) It might possibly result, then, that certain of the middle rows of the twig should never give rise to lateral branches. But I do not believe that this ever occurs in very long rows, for by the splitting up of the twigs the middle rows sooner or later become marginal (so 46–51 XV.). In one stock that I have drawn, consisting of 17 to 21 generations, every middle row occurring as such up to the 13th generation had become at the periphery a marginal row.

As in B. turrita, so in B. flabellata lateral budding occurs most frequently at the margins of fans, — in a fan of about 800 individuals in the ratio of 1 : 10 for the margin, and 1 : 14 for the remainder of the fan. By a comparison of these figures with those given on page 43 for B. turrita, it will also appear that lateral budding is less frequent here relatively to terminal budding than in B. turrita.

The fifth rule deduced for B. turrita holds equally well here. In one case the curve of the tips of the rows rises from the margin of the fan at

[1] The species which I have studied is identified by Verrill ('73, pp. 711, 389) under this name, and my specimens also agree fairly with Hincks's ('80, pp. 80–82) diagnosis. The two pairs of spines, one longer than the other, could be distinctly seen. Hincks says, "The rows of cells . . . are never, I believe, fewer than four, and range as high as seven." But his Figure 66 shows three rows only in some places.

generation XXIV., reaches 3 maxima of XXVI., XXVII., and XXVIII. respectively, and falls again at the other margin to generation XXIII. In the subfan from which Figure 66 was taken, the curve begins at the outer margin with generation XVII., rises to generation XXII. at two points, and falls again to XX. at the inner margin of the subfan.

Of the four proximal individuals in any fan here, as in Bugula turrita, the outermost, ancestral give rise to the greater number of individuals. In one case, for instance, the marginal individuals lie at the base of 31 rows with 184 individuals, while the inner ones support only 7 branches with 65 individuals. Similar results were obtained from other stocks.

With the middle of the primary individual as a centre, I passed an arc of a circle through the extremities of the branches of a large camera drawing of a fan of B. flabellata, divided the radius into eighths, and passed arcs through these points. The number of individuals cut by the different arcs was then counted and tabulated; the arc with the longest radius cut through 87 individuals. By measuring the length of the arcs, the number which should be cut by each arc on the assumption that the number of individuals per unit of arc is constant for all radii was determined. This was then compared with the actual number found, with the following results: —

Length of Radius.	No. of Individuals observed.	Theoretic No.	Length of Radius.	No. of Individuals observed.	Theoretic No.
1	3	2½	5	37	40
2	7	6	6	56	56
3	13	13	7	68	70
4	22	25	8	87	[87]

In this instance, then, the 7th rule deduced for B. turrita evidently holds true for B. flabellata.

While at Mr. Agassiz's laboratory at Newport, during the summer of 1890, I had frequent opportunity to examine other stocks of Bryozoa, which occur there very abundantly. I will take four species as typical examples of the groups they represent, and treat of the architecture of their colonies.

Lepralia Pallasiana, Busk.[1] — It is not at all easy to determine

[1] I do not feel perfectly certain that the specimen shown in Figure 71 (Plate VIII.) belongs to this species, because the characters of the young stocks differ some-

from a young stock what has been the order of succession of individuals. One has to view the object from both sides, make a careful examination of the walls of the zoœcia and of the relation of the polypides to one another, and, when he has done his best to determine what are the facts, he must feel that his conclusions are after all more or less subjective. By a careful study of the colony shown in Figure 71, I have constructed the diagram shown in Figure 71[a].

The stock of Lepralia is a creeping one, and all of its rows of individuals are in juxtaposition. This juxtaposition is continued into the adult stage. Even the young stock begins to show evidence of a quincunx arrangement of individuals. This is less evident in the youngest individuals than in the older part of the stock, and is most evident in old colonies. That there is not here a true dichotomous division of rows of individuals, resulting in the annihilation of the ancestral row and the establishment of two new ones, is evident from a glance at the youngest generation in rows 11, 12, or, better, 2, 3, in which the relation of terminal (11, 3) and lateral (12, 2) individuals is very different. The former continue the ancestral line, the latter establish new rows. Lepralia differs from Bugula in this: that two lateral branches may be given off from the ancestral row in the same generation, as at *B*, *C*, and *a*, *a* (enclosed in circles), Figure 71[a].

In contradistinction to the conditions in Bugula, when only one branch arises, it is not given off towards the axil, but away from it.

The synchronism of the budding process noticed in B. turrita is hardly distinguishable in the adult stock of this species; in the young, however, it is quite marked, and gives to the whole a very symmetrical form. The cleavage of eggs does not proceed by more regular steps. Of the three individuals *a*, *C*, *a* (in circles), which follow *B*, each has given rise to three others, a median and two lateral. From each of the three individuals derived from the two individuals *a*, *a* (in circles) has arisen a lateral branch. Rule 3 is therefore well marked in the young stock of Lepralia.

Rule 4, concerning the greater frequency of lateral budding at the margin, is also exemplified in Lepralia. The ratio of cases of lateral to median budding being 1 : 1 on the margin (rows 1–6 and 15–19) and 1 : 2.8 in the middle (rows 7–14.)

In Bugula, as will be recalled, it was concluded that the marginal

what from those of older ones. Yet it is an Escharine closely allied to Lepralia, and I have seen in some cases the broad-based spine on the proximal border referred to by Verrill as being found in L. Pallasiana.

branches were possessed of fewer generations than the intermediate ones. Since by Rule 2 the lateral branches were given off towards the axils, and the ancestral branches therefore always remained marginal, it resulted that the ancestral branches were the shorter, the lateral branches the longer. But in Lepralia lateral branches are turned away from the axils, and here we find the conditions concerning the relative number of generations in marginal and intermediate rows correspondingly reversed. Thus, in Figure 71ª, the terminal individual *D* of row 10, a median but ancestral row, belongs to generation IV. while the lateral branches 6 and 15 have five generations of polypides. Thus it is true here, as in Bugula, that the ancestral branches are the shorter, the lateral branches the longer (page 44).

That the outer individuals *a*, *a*, of rows 6 and 15, have given rise to more individuals than the inner *C*, is clear without further comment. Finally, since the individuals retain a nearly constant width, the necessity of the rule established for Bugula, — viz. that there is almost exactly the same number of individuals per unit of arc for all radii, — and of its corollary, — that the increase of individuals in sucessive generations undergoes a regular diminution, — is apparent.

Flustrella hispida, Fabricius.[1] — This stock is a very dense corm-like one. The primary individual becomes surrounded on all sides by the younger zoœcia. It is very evident from an inspection of the position of this primary polypide with relation to the periphery, that growth occurs most rapidly on each side and in front of the primary polypide. In making any diagram of such a stock, it is not very difficult to decide upon the origin of the more peripheral individuals of the stock, but it is wellnigh impossible to say with any certainty what are the relations of the individuals of the second generation to those of the first. Barrois ('77, pp. 227–229) has, however, determined this for this species, and my diagram (Fig. 67) is based in part upon his observations. I do not desire to insist that the diagram represents the exact method of growth of the stock. It is an attempt to represent it, founded principally on careful study of Figure 69. The quincunx arrangement of individuals is already apparent in the young stock (Fig. 69); it becomes

[1] Hincks ('80, pp. 504–506) makes the existence of a larval bivalve shell a characteristic of this genus, and therefore I assign to it a very common Alcyonidium-like form which was extremely abundant on Fucus at Newport. F. hispida is the only species of this genus. I found the bivalve shell still adhering to the primary individual of a young colony (Plate VIII. Fig. 69, *o*.). In Verrill's ('73, p. 708) catalogue this species is referred to under the name "Alcyonidium hispidum, Smitt."

more evident in the adult, and when new individuals arise distad to any two, one of the new ones is median (ancestral branch), the other lateral. (So terminal individual of rows 11 and 10 ; 22, 23 ; 43, 44 ; etc.) In the diagram, however, I have not always indicated which is the median and which the lateral branch, for in the older parts of the colony, owing to a shoving of individuals, it is not easy to distinguish them.

Lateral branches appear usually to be given off towards the axis. Here, as in Bugula, the lateral branches tend to be longer ; the ancestral, shorter.

It is evident from the diagram that lateral budding is most frequent at the margins of the corm, i. e. that part lying posterio-dextral or posterio-sinistral of the primary individual, and that the descendants of the two lateral individuals of the four belonging to generation II. are more numerous than those derived from the middle two. Finally, it is evident that the number of individuals per unit of arc will be the same for arcs of all radii, and therefore the rate of increase of individuals will diminish through successive generations.

In *Crisia eburnea*, Linn.,[1] we find the same laws illustrated. The architecture of the genus has been carefully treated of by Smitt ('65[a], pp. 115–142) as forming the basis of classification. Barrois ('77, pp. 76–85) has described in a masterly way the formation of the young stock of tubuliporid Cyclostomata, and the relationships of the different types of budding in this group. Harmer ('91, pp. 145–173) has recently discussed the architecture of the stock in British species, adopting Smitt's graphic method of showing it. I have found his paper of great value for my purpose.

This species grows as a shrub-like stock upon floating eel-grass, etc. I was wrong in saying, in my Preliminary ('91, p. 282), that Crisia has its branches united in pairs. The comparison of this species made by Barrois ('77, p. 82) with the "geniculata form" is conclusive evidence, to my mind, that the apparent double row is in reality a single one, and that such a branch as 18, Figure 65, is to be represented by a single line in the diagram Figure 65[a]. We find here terminal and lateral branches ; no true dichotomy. Branches are given off on the side *away* from the axils, as in Lepralia, not as in Bugula. (But branch 11 is an exception to the rule.) They are given off, as Harmer ('91, p. 131) has shown, alternately to the right and left.

[1] This is the only species of Crisia given by Verrill, and, since my species is very common, it must be the one to which he refers. Moreover, it agrees fairly well with Harmer's diagnosis ('91, p. 131).

There is something of a tendency for lateral branches to be given off in the same generation from closely related branches. Thus (Fig. 65[a]) from the primary individual, *o*, of the stock, two individuals, a median and a lateral one, arise. Each gives rise in its first generation to two individuals, a median and a lateral. Of these four individuals each gives rise at the end of three generations of median buds to two buds, a median and a lateral. Comparing 2 and 10, first descendants of the two branches arising from the second individual of 8, we find that each gives rise to lateral branches from their first individual and from their fourth. Comparing 14 and 19, first descendants of the two branches arising from the first individual of 8, we find each giving rise to lateral branches from their first individuals. The law breaks down, however, when an attempt is made to carry it to extremes.

The fourth rule is not always so pronounced in Crisia eburnea as elsewhere, although lateral budding seems to be slightly more frequent at the margin.

The extreme marginal branches usually attain far fewer generations than the more intermediate ones; thus, in Figure 65[a], branch 20 ends in the 7th generation and branch 13 in the 7th also, while the more intermediate branches 15 and 18 attain 12 and 14 generations respectively. So, too, while the outer branches 6 and 1 contain respectively 10 and 11 generations, the inner branches reach 12 and 14.

It is very noticeable that the outer branches give rise to more individuals than the intermediate ones. Figure 65[a] will serve to illustrate this also. Here the outer branch 4, the intermediate 8, and the outer 15 possess, together with the branches arising from them, 33, 28, and 40 individuals respectively. Harmer ('91, p. 168) finds this true for his Crisia ramosa, for he says, "It is frequently remarked that the longest and most branched parts of the colony are lateral branches, and not parts of the main stems."

There is, in the long run, a decrement in the rate of increase of individuals in successively older generations, yet it is not so regular a one as that which we found to exist in Bugula. Thus, in the seven generations which even the shortest branches shown in Figure 65[a] had attained, the average increase of the number of individuals in the second, third, and fourth generations over the number in the preceding is 67%; in the fifth, sixth, and seventh, 44%. The generations beyond the seventh are not complete; they would have contained more individuals at a later period, when the branches which have now attained only seven generations had grown. Thus the number of individuals

in successive generations beyond the seventh increases more and more slowly, and finally decreases to zero. Thus the average rate of increase of individuals in the generations 7 to 10 over those in the preceding is only 16%.

One finds here, as elsewhere, that the number of individuals cut by any unit of arc, the primary individual being taken as a centre, remains practically constant, whatever the radius of the arc.

In studying the creeping stocks of Cheilostomes (Plate VIII. Fig. 71), young corms have been chosen because they exhibit fewer irregularities of formation than old ones. Such irregularities are chiefly due to some unevenness of the surface on which the corms lie, but sometimes apparently to a crowding of individuals. Old rows of individuals are occasionally entirely cut off and end in the middle of the stock; sometimes two rows running side by side, perhaps derived from a common ancestor, suddenly merge into one again. In one case, Escharella variabilis, Verrill, I have seen three rows thus merge into one at the margin, suggesting the existence of a *samknopp* (common bud) in the sense of Smitt ('65, pp. 5–16). Ostroumoff ('86[a], pp. 338, 339) has observed a case in Lepralia Pallasiana. He says: "Dans quelques cas, qu'on peut considérer comme des anomalies, il arrive parfois que deux bourgeons, provenant de loges différentes, viennent à se fusionner." It seems to me, therefore, that while Nitsche ('71, pp. 445, 446), who opposed with such vehemence and success the idea of Smitt that zoœcia arise from an undivided marginal zone of cells, was quite right in affirming ('71, p. 447) that even the smallest marginal zoœcia are sharply marked off from the adjacent ones, yet he overlooked the possibility that under certain circumstances the lateral walls might fail to develop, and thus one zoœcium might arise in the place of two, or even three.

I have not read Smitt's Swedish paper, but I do not find anything in the translation given by Nitsche to warrant the latter's conclusion ('71, p. 446) that Smitt believed the "Gesammtknospe" to be "formed from the sum total of the mature peripheral zoœcia." If I understand Smitt, he conceived the *samknopp* not to be derived from the most peripheral mature zoœcia, but to be self-proliferating, and to give rise to the rows of zoœcia, not to arise from them. It is the "bud of the colony," not the sum of the buds of the peripheral individuals of the stock. In this I would agree with him exactly. Although *usually* one finds the marginal gemmiparous tissue forming the lateral walls at the extreme edge of the corm, and thus apparently separated into wholly distinct adjacent gemmiparous masses; under certain conditions, the

lateral wall may not be formed between two or more rows, which will then merge into one.

2. Origin and Development of the Individual.

My studies on this subject, which were undertaken for the purpose of showing the unity of the type of budding throughout Ectoprocta, have been very fragmentary.

Figure 72 (Plate IX.) has been introduced for the sake of orientation. It represents a longitudinal vertical section through the peripheral part of a stock of Lepralia Pallasiana. The body wall is thicker at the margin (*marg.*), and gradually becomes thinner as one passes backward. A septum (*sep.*) has already arisen cutting off the youngest zoœcium from the more proximal one, which contains a young polypide; proximal to this is another septum, and the distal end of a third zoœcium.

Nitsche ('71, pp. 445–456) has already well described the process of forming the *zoœcium* in Flustra membranacea. In fact, he has studied the organogeny more thoroughly in many respects than I have. Nitsche ('71, p. 452) showed that the wall of the advancing margin of the colony was composed of two layers of cells, — an outer, "Cylinderepithelschicht," which secretes a cuticula, and an inner, "Spindelzellschicht mit anliegenden Körnerhaufen." As the body wall formed directly from these cell layers is left behind by the advance of the margin, it becomes continually thinner. "Die Cylinderepithelzellen der Wandung platten sich weiter nach dem proximalen Ende zu ein wenig ab, besonders die der Unterseite verkürzen sich, die einzelnen Zellen rücken auseinander, die Zellgrenzen werden undeutlicher, die Kerne jedoch bleiben deutlich erkennbar." Vigelius ('84, p. 76) could not find the inner cell layer in Flustra, even at the youngest stages, and consequently he believed that only one existed at the margin, and that this went to form the "*Parenchymgewebe*" of the adult. Ostroumoff ('86[a], p. 336) seems inclined to doubt the existence of any mesodermal *layer* at the distal portion of the budding zoœcium in Cheilostomes, and Seeliger ('90, p. 580) has failed to find in Bugula "eine *zusammenhängende* dem Ectoderm dicht anliegende Schicht von mesodermalen Spindelzellen." Both Ostroumoff and Seeliger, however, believe in the existence of *isolated* mesodermal elements at the budding end.

According to my own observations, there is usually only one continuous layer at the budding margin of the stock. Thus, in Flustrella (Plate IX. Fig. 79) one can usually distinguish a continuous ectoderm, but the mesoderm (*ms'drm.*) is represented by scattered cells only. At

the margin in Lepralia (Fig. 73) one finds a thick ectodermal layer, composed of columnar cells, but the mesoderm consists of an irregular thick mass of cells, some of which appear to be amœboid. They however show no signs of having been derived from the outer layer. The condition of the budding margin of Escharella resembles that of Lepralia. In older parts of the body wall, where the ectoderm is reduced to an extremely thin layer, only scattered mesodermal cells appear, and these are amœboid or mesenchymatoid.

On the other hand, one finds in the body wall, around the nascent neck of the polypide (Plate X. Fig. 88), even to a late stage, both ectoderm and mesoderm well formed as *layers*. The ectoderm is a columnar epithelium; the mesoderm is flatter, and often its cells are not sharply delimited from one another. It is thus perfectly evident, to my mind, that the mesoderm has in general lost its original epithelial character in the marine Bryozoa, although it has retained it in Phylactolæmata. Whenever it does exist in the former group as an epithelium, it is at the budding regions (neck of polypide, and Figures 74, 75, 78, 79, *ex.*).

Origin of the Polypide. — There are very few problems in modern morphology, I fancy, the history of whose investigation shows a less satisfactory aspect than that of the origin of the polypide in Gymnolæmata. It is hardly to be wondered, however, that investigators have sought for another interpretation of the process than the most obvious one, because that seemed to oppose many long cherished and wellnigh universally held dogmas. While the first recognition of the animal nature of marine Bryozoa, which we owe to the studies of Bernard de Jussieu in 1742 and John Ellis in 1755, brought with it a knowledge of their colonial nature, yet it was not until much later that the most characteristic part of this process — the formation of the polypide — was clearly observed. Grant ('27, p. 115) and Farre ('37, pp. 400, 409, 415) first described the process by which is formed this complex of organs, and settled once for all the controversy which had sprung up as to whether these animals were truly stock-builders. Under the influence on the one hand of the endosarc theory of Joliet ('77), and on the other hand of the view promulgated by Hatschek ('77), that similar organs in larva and polypide are equivalent as far as regards their origin from the germ layers, the more important papers [1] between '77 and '90 maintained either that the polypide arose independently of the body wall,

[1] Excepting those of Barrois, who, from the study of the favorable material presented by metamorphosing larvæ, has persistently maintained the correct interpretation.

and secondarily acquired connection with it, or that it had a double origin.

To Nitsche ('71, pp. 456–463) belongs the credit of having first described the histological changes in the origin and development of the polypide of marine Bryozoa, particularly with reference to the part which the germ layers play in that process. He says ('71, p. 456): "Die Anlage des Polypids erscheint zunächst als eine Wucherung der Zellschicht der Endocyste in der Mitte der Hinterwand der Knospe, und zwar in dem Winkel, den die Hinterwand mit der oberen Wand macht. Bald ordnen sich die Bestandtheile des regellosen Zellhaufens in zwei deutlich gesonderte Schichten, und wir sehen nun einen rundlichen Körper, bestehend aus einer äusseren einschichtigen Zellschicht, welche sich scharf absetzt gegen die das Innere des Körpers bildenden Zellen."

This stood until a year ago as the most satisfactory description of this process in the adult stock. The appearance within the last year of the two papers of Prouho ('90) and Seeliger ('90) marks a distinct epoch in the advance of our knowledge concerning the origin of the polypide in Gymnolæmata. The paper of Prouho treats of the process in the case of the primary polypide of the metamorphosing larva of Flustrella, that of Seeliger in the case of the young (practically adult) stock of Bugula. According to both authors, the polypide arises from the body wall by an invagination of it, and its two layers are from the first distinct and separate, and go to form the two layers of the adult polypide, and the whole of those two layers. The outer layer of the body wall gives rise to the outer layer of the tentacles and the lining of the alimentary tract, and the inner layer of the body wall gives rise to the mesodermal lining of the polypide. Prouho alone is cognizant of the method of origin of the ganglion, and in addition there are several points of difference between these two authors concerning the development of other organs, to which I shall refer in the proper place. Thus the latest studies have confirmed the assertions of Nitsche, that the polypide arises from a single centre of proliferation of the body wall; they have made an advance in this, that they have shown that the two layers of the bud do not become secondarily differentiated from a single cell mass, but are respectively derived from the two cell layers of the body wall. My own studies have led me to the same conclusion on this point.

Figure 75 (Plate IX.) is a vertical radial section through the margin of an adult Flustrella stock. The ectoderm is relatively thick at the sole (*sol.*) and margin, and very greatly thickened at the point marked *gm*. Here two layers, sharply separated, are apparent. The cells of the outer

layer are columnar and full of granular protoplasm, the mesodermal cells cuboid. The body wall has clearly begun to invaginate in this region. Figure 79 is a similar section, and shows a later stage in this process. The lumen of the bud is apparent, and has been formed by invagination, not, as in Paludicella or Phylactolæmata, by ingression. The two layers of the bud are apparent; they have been derived from those of the body wall.

Figure 73 (Plate IX.) shows a stage in the development of the polypide which is intermediate between that of Figures 75 and 79, but from another suborder, Cheilostomata. The mesoderm has here a mesenchymatous character, and is loosely attached to the inner layer of the bud; it is not always sharply marked off from it by boundaries, but is quite distinct in its reaction with staining reagents. This bud has evidently arisen by invagination of the body wall. Seeliger ('90, p. 581) also finds that there is an actual invagination of the ectoderm in Bugula, the opening to which he calls "blastopore."

From what has been already shown, it is evident that in Flustrella, as well as in Cheilostomata, the first appearance of the young polypide is near the margin of the stock, not near the proximal part of the young zoœcium. This will also be apparent at 6 and 9, Figure 71 (Plate VIII.), where the accumulation of nuclei immediately behind the margin indicates the neck of the polypide, — the point at which the bud arose. To be sure, at quite an early stage, but very much later than that of Figure 73, the polypides are found near the proximal wall of the zoœcium, but a delicate funnel-shaped sheath of tissue runs from the polypide to the distal part of the zoœcium, where the polypide is attached to the body wall.

After invagination the pocket closes at its attached end by a growing together of its lips (Figs. 79, 78). Thus the body wall becomes continuous again over the lumen of the bud, and this union is first broken when the fully formed polypide is ready to evaginate itself. Seeliger ('90, p. 582, Taf. XXVI. Figs. 8, 10) has described and figured a similar condition in Bugula.

The young bud now becomes elongated (Fig. 80), the walls of the bud sometimes becoming closely approximated. A little later it begins to pass backwards relatively to the distal wall of the zoœcium. A transverse section through the young polypide and the neck of the colony shows that the connection has become a less intimate one (Fig. 81, *cev. pyd.*). The tissue by which the connection is still effected is that from which the kamptoderm will be formed. It is apparently the existence

of this stage, in which the kamptoderm is long drawn out and easily overlooked in optical as well as actual sections, that led to the belief that polypide buds may arise independently of the body wall and only secondarily become connected with it.

At about this time the lumen of the *alimentary tract* begins to be separated from that of the atrium. Thus, in the series from which Figure 81 was taken the more oralward lying sections show that the cavities of the lower and the upper parts of the bud, which at the anal end are broadly confluent, have here become separated by a constriction. A sagittal section of a somewhat later stage is shown in Figure 76, which is from Flustrella. Here we find the alimentary tract represented by a space in the lower part of the bud, broader at its anal than at its oral end and separated from the upper cavity — the common atrio-pharyngeal cavity, *œ.* + *atr.* — by a line of nuclei which represents the line of approximation of the inner layers of the two sides of the bud. The bud is attached to the body wall at its marginal (anal) end, and is free from it oralwards. (Compare with Paludicella, Plate III. Fig. 24.) It seems to me highly probable from these and other series of sections that the alimentary tract is separated from the rest of the lumen of the bud, not by an approximation of the inner layers of the bud along the whole extent of the future alimentary tract at once, but that the rectal part is first formed and constitutes a large cavity, at first broadly open to the atrium above, and that the gastric portion is formed somewhat later by a progressive enlargement of the lower cavity of the bud, which now becomes constricted off from the atrium and œsophagus above. This process is like that found in Paludicella (page 19), which forms a sort of transition to that of Phylactolæmata, described by Braem ('90, pp. 45, 46) and myself ('90, p. 112).

Prouho ('90, p. 448, Fig. 6) shows that the rectum at first appears as a *blind sac* open to the atrium at its posterior end, although later this opening is greatly reduced. Hence in the Flustrella larva also the space from which the lumen of the future rectum is to arise is formed before that of the stomach, although this part of the alimentary tract is the last to be cut off from the atrium. Seeliger ('90, p. 585) says concerning the formation of the alimentary tract in Bugula : "Der ganze Basaltheil des Polypids sich in der Mittelpartie durch zwei immer tiefer werdende Furchen von dem vorderen abschnürt, während er an zwei Stellen, einer oberen und einer unteren, mit ihm in Verbindung bleibt. Die obere Verbindung entspricht dem Anus, die untere dem Mund." The author here seems to imply that the whole alimentary tract is formed at

one time; but as he has not attended particularly to this point, this can hardly be said to militate against my view.

There is, however, in my opinion, a more important error in Seeliger's description of the origin of the alimentary tract, — an error into which Nitsche ('71, p. 457) also fell. As in Phylactolæmata and Paludicella, so also in marine Bryozoa in general, so far as I have studied them, the posterior and anterior parts of the alimentary tract are formed independently, and their cavities coalesce only secondarily. The constriction which separates the lumen of the bud into a cavity nearer, "*vorder*," and one more remote from the body wall, "*basal*," does not separate off the whole alimentary tract from the atrium. Neither does that constriction result in the formation of a space opening into the cavity nearer the body wall, "*Vordertheil*," at an upper [distal] point (anus) and lower [proximal] point (mouth). Thus if one examines a complete series of sections through a polypide even of so late a stage as Figure 92 (Plate X.), one finds that, while there is an open connection between the anal end of the alimentary tract and the atrium, the oral end is at all points sharply separated from the cavity above by a double-layered wall of cells, as is shown in Figure 92, between *æ.* and *ga.* Such a condition, moreover, has been found by Barrois ('86, pp. 73–76) in the primary polypide of Lepralia, and by Prouho, as just stated, in the primary polypide of Flustrella.

Origin and Development of the Ring Canal and Tentacles. — Nitsche ('71, p. 430) first described in Flustra a ring canal surrounding the mouth-opening and lying at the base of the tentacles, but did not refer to the origin of it. Seeliger ('90, p. 588) describes it in a young polypide of Bugula, as derived from the mesodermal layer.

My own sections also show that it arises on each side of the œsophagus as a groove lined by mesoderm (Plate X. Fig. 92, right). This canal, which is shown cut along its course in Plate IX. Fig. 82, *can. crc.*, is not wholly separated from the body cavity, but communicates with it below the brain. This communication occurs in the section below that shown in Figure 82, near the point *can. crc.* This ring canal at an earlier stage is shown in Figure 87. It has not yet been formed backwards nearly so far as the brain; anteriorly the section has traversed the tentacles under which it runs. The canal is also shown cut across in Figure 86 at the base of a tentacle, with whose lumen its cavity is directly continuous.

The formation of the tentacles is closely connected with that of the ring canal, from the upper wall of which they arise. Since the upper

wall of the ring canal is two-layered, the tentacles are two-layered also. The outer layer of the tentacle is thus derived from the inner layer of the bud; the inner layer, on the contrary, from the outer layer of the bud. It would be hardly necessary to make this statement, which agrees both with early and the most recent observations, had not Barrois ('86, p. 75, Fig. 48) referred to and figured the tentacles as having been formed from the inner layer of the bud *only*.

My observations fully confirm Seeliger's ('90, p. 587) description of the manner of growth of the tentacles; that is, that the outer edge of the ring canal, together with its tentacles, moves downward and outward along the sides of the polypide, turning the axis of the tentacle from a nearly horizontal to a vertical position, and increasing the area of the kamptoderm. Thus in Figure 92 this process has progressed farther on the left side than it has on the right.

Nitsche ('71, p. 458) lays some stress upon the statement that the tentacles are not at first few in number, gradually becoming more numerous; on the contrary, he says, "Ich sah stets, beim ersten Auftreten von Tentakelanlagen, 16, 17, oder 18 Stück gleichtzeitig erscheinen." Seeliger ('90, p. 584) agrees with Nitsche in this respect; but Prouho ('90, p. 449) finds the conditions different in Flustrella. Here the tentacles "ne se développent pas simultanément sur tout son pourtour, mais apparaissent d'abord de chaque côté du plan de symétrie, puis se multiplient vers l'arrière." As I have shown, 14 of the 17 tentacles arise nearly simultaneously in Paludicella, for here there are few of them; and this is the case also in Escharella variabilis with its 17 tentacles.

As the tentacles of both Flustra and Bugula are few in number,[1] the statements may easily be considered to be correct for these genera. The tentacles of Flustrella hispida are much more numerous (30–35), and Prouho's statement may well be true for his form. In fact, my own observations on this species are fully in accord with those of Prouho. Figure 77 (Plate IX.) represents a young polypide of a Flustrella corm, viewed from the roof as an opaque object. Six tentacles were visible on each side of the bud, but the oral and anal parts of the corona were yet incomplete. The remaining nine or ten pairs of tentacles subsequently arise oralward and analward of these rudiments.

Much disagreement has prevailed concerning the number of layers involved in the *kamptoderm* of marine Gymnolæmata, in both the adult and the developmental stages. As in so many other cases, we owe to

[1] Bugula avicularia has 14 or 15 tentacles, and Flustra (Membranipora) membranacea 20, according to Hincks ('80, pp. 76 and 140).

Nitsche ('71, pp. 431, 432) our first intimate knowledge of this organ. He believed it to consist in the adult of Flustra of a single cell layer, in which are imbedded (or applied?) longitudinal and circular muscle fibres. He believed the kamptoderm to be formed gemmigenetically only by the outer cell layer, the derivative of the mesoderm. Repiachoff ('75, pp. 138, 139) observed in Tendra (Membranipora) "die Doppelschichtigkeit der Tentakelscheide nicht nur bei den jungen Knospe sondern auch bei den ganz ausgewachsenen, offenbar schon längst functionirenden, in ihrem mittleren Theile ganz braunen 'Polypiden,'" and later ('76, p. 152) a similar two-layered condition of the kamptoderm (Tentakelscheide) in Membranipora and Lepralia. Ehlers ('76, p. 37) finds a single layer of cells in the kamptoderm of the adult Hypophorella (Ctenostome), which he believes is continuous with the endocyst of the body wall, and thus is ectodermal. He finds neither longitudinal nor circular muscle fibres. Haddon ('83, p. 517) believes the kamptoderm to be derived from both the inner and outer layer of the polypide bud. Vigelius ('84, pp. 33, 82) describes it as arising from the mesoderm only (Parenchymegewebe), and as being essentially one-layered, both longitudinal and circular muscles lying in this layer. Barrois ('86, p. 74) derives the kamptoderm from the mesodermal layer only. Ostroumoff ('86[a], p. 15) believes the kamptoderm to be two-layered and provided with muscles; it is in his opinion derived from both layers of the bud. Freese ('88, pp. 18, 19) studied only the adult of Membranipora. He admits the presence of muscle fibres, but believes the kamptoderm one-layered. Pergens ('89, p. 507) states only that in the Cheilostomes studied by him the tissue of the kamptoderm is composed "aus abgeplatteten Zellen, zwischen welchen Längs- und Ringmuskelfasern eingebettet sind." Prouho ('90, p. 451) states that in the primary polypide of Flustrella this organ is early differentiated, "et les deux couches de rudiment prennent part à sa formation." Finally, Seeliger ('90, p. 587): "Es kann danach keinem Zweifel unterliegen, dass die Tentakelscheide ektodermalen Ursprungs ist. . . . Das Mesoderm erscheint auf allen gelungenen Schnitten von der Tentakelscheide scharf abgesetzt."

It is my belief that throughout the group of marine Gymnolæmata, as in Paludicella and Phylactolæmata, the kamptoderm is derived from both of the two layers of the polypide bud, is provided with a strong system of longitudinal and a slight one of circular muscles, and contains in the adult two layers, or at least modified representatives of two layers. I have arrived at this conclusion from a careful study by sections of the following genera: Bugula, Lepralia, Escharella, Flustrella, Bowerbankia,

and Crisia. The existence of two layers was easily demonstrated in all cases in the young polypide by cross sections of the "neck." The two layers are of nearly the same thickness, and distinctly separated from each other. The presence of two layers in the adult is more difficult to determine, but it was always indicated by the occasional presence of two nuclei lying side by side, and especially at the attachment to the diaphragm. The presence of muscles was demonstrated in all cases (except Bowerbankia, where my few sections did not show the proper region) upon tangential sections of the sheath. I may add, that the existence of muscles is wellnigh conclusive *a priori* evidence of the existence of the mesodermal layer, since nowhere else in Bryozoa, so far as I know, do muscles arise from any other layer. Prouho's evidence in support of his position is perfectly satisfactory to my mind, certainly more so than the negative evidence of Seeliger in support of his. In further support of my statements I may refer to the condition of the kamptoderm (*kmp'drm.*) in Figures 92 and 83, Plate X.

Nervous System. — Since Dumortier discovered, in 1835, a ganglion in Lophopus, there has been seen in marine as well as fresh water Bryozoa a body which has been considered, with greater or less certainty, to constitute the central nervous system. Overlooked by Farre, it was, I believe, first described for marine Gymnolæmata in 1845 by van Beneden, co-worker with Dumortier, for Laguncula (Farrella). Nevertheless, up to the present the evidence of its being a ganglion homologous with that of Phylactolæmata has not been satisfactory. The homology can be established only by determining its similar origin with the brain of Phylactolæmata; its function can be best established by showing the existence of ganglionic cells and fibres. I hope to have advanced our knowledge in both of these directions.

At about the time that the œsophagus and stomach have become confluent, one notices a papilla-like elevation of the floor of the atrio-pharyngeal cavity. This has been noticed by Korotneff ('74) in Paludicella, and by Nitsche ('71, p. 459) and Seeliger ('90, p. 586) in Cheilostomes. It has been called by them "Epistome," and compared with that of Endoprocta or Phylactolæmata. In my own opinion, it is merely a structure brought into prominence by the sinking down of the floor behind it to form the ganglion (Plate X. Fig. 86, *gn.*). This depression has been seen by Barrois ('86, pp. 74, 75) and Prouho ('90, p. 450), and rightly interpreted by them as probably destined to give rise to the central nervous system. That this is the correct interpretation is shown by later stages from different species, as Figures 89 and 83, in which we see

the ganglion gradually assuming the position it has in the adult, on the anal side of the pharynx at the base of the anal tentacles.

A section across the pharynx in such a stage as Figure 83 is shown in Figure 87. A comparison with Figure 51 (Plate V.) of my Cristatella paper (Davenport, '90) will show a great similarity of conditions at about the same age, and can leave no doubt concerning the homology of the regions marked in both cases *lu. gm.*; or compare Taf. VIII. Fig. 100, *nh.*, of Braem's ('90) magnificent work. A section through a later stage is shown in Figure 82. The brain has already sent out circumœsophageal nerves, as in Paludicella. The central part of the ganglion does not stain; one sees only a granular mass, sometimes with signs of short fibres. In the cornua (n') one occasionally sees very large clear nuclei with a single nucleolus, lying in the midst of a cell mass which is spindle-shaped and stains more deeply than adjacent cells. These remind one strongly of bipolar ganglionic cells, but fibres could not be traced far from their pointed ends. Series of sections of Flustrella parallel to Figure 82 show, as one passes below the level of the ganglion, a continuous band of cells extending down from it towards the cardiac valve and between the cell layer lining the œsophagus and the surrounding mesoderm. One is reminded of the exactly similar conditions in Paludicella (page 26), and of the "linienartige Zeichnung" seen by Nitsche ('71, p. 431) and Vigelius ('84, p. 42) in the same place in Flustra. These facts go to indicate the existence of a gastric nerve.

At about the time at which the ganglion arises, the cavities of the stomach and the œsophagus become confluent (Fig. 86 *œ.*). At this stage (somewhat earlier than Figure 86) the alimentary tract consists of a U-shaped tube of nearly uniform calibre, and without any indication of the cœcum. The tentacles lie in two parallel rows in the middle of the bud, the corona being incomplete both in front and behind, but less so oralwards than towards the anus (Fig. 77, *atr.*). In fact, while new tentacles are formed later towards the oral median line, they never appear behind the line *atr.* This hinder region has another fate. Its wall increases very greatly in area, diminishes correspondingly in thickness, and forms a large part of the kamptoderm lying behind the postoral tentacle in Figure 86. With this growth of the kamptoderm the anus is carried backwards, and farther and farther from the posterior ends of the rows of tentacles, immediately behind which it formerly lay.

As the kamptoderm grows in area, the polypide comes to lie in the proximal part of the zoœcium. *Pari passu* with this process occurs the *rotation of the oral tentacles*, as in Paludicella. The oral tentacles which

at first lie perpendicular to the roof of the colony (Fig. 86) gradually come to lie parallel with it (Figs. 89 and 83). The œsophagus loses its elongated, laterally compressed form, and becomes circular, and the ganglion lies just below the mouth-opening. Not until now, in fact, can one speak of a mouth. It was not at all formed synchronously with the anus. To illustrate this process I have taken three different genera representing different stages. Similar stages could have been obtained from each genus. By using three genera, the similarities as well as the dissimilarities of the process are indicated. Among other things, the larger size of the polypide and shorter kamptoderm of the Ctenostome Flustrella (Fig. 89) is noticeable.

Lastly, the cœcum is formed as a wholly secondary differentiation of the alimentary tract. This arises in some species relatively earlier than in others; thus it is better developed in Figure 86 than in the later stage of Figure 83.

The lining cells of the alimentary tract now rapidly undergo the differentiations characteristic of the different regions. The most extreme modification takes place in the *pharynx*. In Cheilostomes the cells of this region gradually become vacuolated, until finally very little stainable protoplasma remains. The nucleus lies at the deep end of the cells. A very peculiar modification of the cell walls takes place, in that they become plainly perforated by holes through which the adjacent cells are in communication (Fig. 85). It is in a region similar to this that the cells become cuticularized in Bowerbankia to form the so-called gizzard. The pharyngeo-œsophageal region is also provided with a very powerful musculature of circular muscles (*mu.*, Figs. 85, 86).

Concerning the *origin of the muscles* I have made very few studies. The parieto-vaginal muscles seem to arise, as in Paludicella, from around the neck of the polypide, and the retractors from the oral end of the polypide bud (*mu. ret.*, Fig. 89).

The *neck of the polypide* sinks below the general level of the body wall by an infolding of the latter, as described for Paludicella, and the mass of columnar cells which passes down with it forms, I am confident, the *diaphragma* of Nitsche ('71, p. 432), which is thus exactly comparable with the mass of cells around the atrial opening of Paludicella in Figure 45, *of. atr.* (Plate V.). According to this view, then, the diaphragma is not placed at about the middle of the kamptoderm, but at its proximal end, and all that lies between it and the outer body wall — the non-evaginable portion — has been formed in the elongated neck, exactly as the non-evaginable portion is formed in Phylactolæmata (see

Davenport, '90, Plate IX. Fig. 77, Plate XI. Fig. 98) and Paludicella (Plate V. Figs. 50 and 45).

As my purpose is not so much to present a complete organogeny of Bryozoa as to show the method of origin of the bud and the fate of the layers, I have had to desist from carrying on my studies further in the organography, and have left many interesting and important questions unsolved; such, for instance, as the development and structure of avicularia, the presence of an excretory system, and the degenerative processes which occur with regularity in the polypides.

3. Regeneration of the Polypide.

I have been led to study the regeneration of the polypide because Ostroumoff seems to believe that in regenerating buds the digestive epithelium of the stomach is derived from an extraneous source, — the brown body. Thus he says ('86[a], p. 340) the brown body appears as a cœcal appendage of the young digestive tube. "C'est sur ce dernier [tube digestif] qu'on trouve un groupe de cellules affectant la forme d'un bonnet et se réunissant très tôt à l'angle proximal du rudiment ectodermique. A mesure que les cellules du bonnet, ainsi que la masse brune, sont employées à la formation de la portion moyenne du tube digestif, ces dernières se débarrassent de leur contenu," etc.

The external phenomena of regeneration are well known. In the Membranipora stock, for instance, one sees polypides being produced at the margin, and one finds them older and older as one passes backwards, until finally they are seen to be wholly degenerate, and to be replaced by young polypides. Thus, in passing backward along a single row of individuals in a Membranipora stock about 18 mm. long, I have seen this process of regeneration recurring four times. In Alcyonidiun, too, one finds an apparently regularly recurring degeneration and regeneration of polypides. In the mat-like Cheilostomata the regenerating polypide (Plate VIII. Fig. 71, *pyd. rgn.*) is always found at one place, — namely, on the operculum, — that is, proximal of the opercular opening.[1] In Flustrella it is found in a similar position on the dorsal body wall, proximal of the cuticularized introverted portion. My studies have been chiefly made on the Cheilostomata. Figure 91 (Plate X.) represents an early stage in the formation of a regenerating polypide. Here, as in the marginal polypides, there is a typical invagination involving the two

[1] Haddon ('83, pp. 522, 523) has found the regenerating polypide arising from the same place in Flustra membranacea and in Eucratea, and Ostroumoff ('86[a], p 339) in Cheilostomes in general.

layers of the body wall (*i.*, *ex.*). Owing to the reagent, the body wall is shrunken from its contact with the operculum (*op.*).

If one inquires what has been the histological conditions of this region antecedent to this stage, one must look to younger adjacent and marginal zoœcia, since they reproduce these conditions. I will again call attention to Figure 88, which represents a cross section of the body wall through the region of attachment of the kamptoderm of a young polypide of about the stage of Figure 83. This, then, represents the neck of the polypide, and it is from about this region that the operculum and finally the regenerating polypides will arise. The cells are columnar, and stain deeply about the nuclei, and both cell layers are well developed. Elsewhere in this same individual the body wall is composed of smaller, flatter cells, and two layers are not easily distinguished. The region of the future operculum possesses at an early stage some of the largest, most columnar cells of the body wall. The cells of this region do not, however, retain their peculiarly large size throughout life, but in the adult we find the same region occupied by a flat epithelium, nearly as thin as the epithelium shown in Figure 90. Meanwhile the epithelium of the rest of the body wall has become still more attenuated. The difference between the body wall of the operculum and that of adjacent regions is best shown by the greater abundance of nuclei under the opercular region when the stained stock is looked at *in toto* from the roof (Plate VIII. Fig. 71). The regions of the future opercula are seen, in young zoœcia (Fig. 71, *4*, *6*), to be patches of densely packed nuclei. The opercula of older zoœcia show a slight preponderance of nuclei, and thus indicate more numerous cells. It is from such a region, then, that the young regenerating polypide arises.

As in the case of the marginal polypides, so here, the lips of the invagination pocket close and become fused to form the neck of the polypide (Plate X. Fig. 84). The later stages of the development of the regenerating polypides seem to be the same as those of the marginal buds. Figures 74 and 89 are, indeed, regenerating polypides. I cannot find any evidence that the alimentary tract, or any part of it, is formed in regenerating buds by a method differing in any essential particular from that in marginal buds.

It is well known, however, that the degenerated polypide which forms a "brown body" in the old zoœcium eventually disappears. Haddon ('83, p. 519) maintains that in the developing regenerated polypide "the walls of the stomach, or, more strictly, that portion of the stomach

which forms the gastric cœcum, grow round and envelop the brown body, so that the brown body passes as a whole into the alimentary tract of the young Flustra." It seems to me that the burden of proof of such a remarkable occurrence lies with him who asserts its existence, and certainly sufficient evidence is not presented by Haddon.

To settle this question in my own mind, I cut a series of thin sections through a part of a stock of Escharella (which in budding shows a practical identity with Flustra), in which all stages of regenerating polypides were to be found. From complete series, at critical ages, I utterly failed to find any indication of the inclusion *in toto* of the brown mass by the polypide. But I found the alimentary tract of the polypides usually applied to the brown body (*pyd. dgn.*), as shown in Figure 92. At this stage the degenerated mass is surrounded by spindle-shaped cells, and just within these by a homogeneous or lamellated sheath. At later stages the elements of the degenerated mass were seen to be more loosely associated. The cells of the alimentary tract at the same time appear highly granular, and a granular coagulum often partly fills the alimentary tract. Before the new polypide is ready to expand itself, the brown body as such has often wholly disappeared. Just as my sections leave no chance for the brown body to be included *en masse* by the alimentary tract, so too do they yield no evidence of the addition to the latter of new cells from this degenerate mass, as Ostroumoff, in the sentence quoted above, implies.

The interesting facts of degeneration in Bryozoa deserve a more careful study than I have been able to give them. We are quite ignorant of the physiological significance of the regularly recurring degeneration and regeneration in certain Bryozoan colonies. Ostroumoff ('86[a], p. 339) has offered an interesting hypothesis, to the effect that the degeneration of the polypides, the remains of which are taken into the stomach of the regenerated polypide and the undigested portion of which is cast out with the fæces, is a method of excretion, made necessary to these animals from lack of urinary tubules.

IV. Origin of the Gemmiparous Tissue in Phylactolæmata.

After having found that in Paludicella and the marine Bryozoa, as in Phylactolæmata, the growth of the colony takes place at the margin or tips, and that it is here primarily that buds originate, and after having thus found that throughout the group all of the organs of the polypide are derived from two layers, of which the inner gives rise to organs so

dissimilar in origin as the central nervous system and the alimentary tract usually are, it becomes a matter of no little importance to solve the two problems, what is the origin of these growing regions, and what that of the two layers. Through the works of Barrois ('86), Ostroumoff ('87), Vigelius ('88), and especially Prouho ('90), on the metamorphosis of the larva and formation of the first polypide of Gymnolæmata we are fairly well acquainted with the facts in this group; but a careful study has not heretofore been made of the Phylactolæmata with reference to the points mentioned above. Korotneff ('89) and Jullien ('90) have published quite extensive papers on the ontogeny of Phylactolæmata, which describe too incompletely the stages which should reveal the required facts.

In order to throw a little light on these questions, I undertook the study of the embryology of two species of Phylactolæmata. But before beginning the account of what I have found, it is necessary to remind the reader of some facts concerning the origin of the polypides in the adult colonies. For our knowledge of these we are chiefly indebted to Braem ('90, pp. 18–32) ; it has also been my privilege to confirm many of them.

The details of the budding process are slightly different in Plumatella and Cristatella. In the latter genus the body wall becomes highly modified as it grows older by the formation of secreted masses which nearly fill most of the ectodermal cells. In Plumatella, on the contrary, the ectodermal cells retain, for the most part, a more primitive, unmodified condition. Here, moreover, by a rapid growth at the neck of the polypides, the individuals are carried to considerable distances from one another, whereas in Cristatella there is a less rapid growth resulting in a compact stock.

In Plumatella, the whole of the embryonic tissue from which any bud arises does not go to the formation of a polypide, but a part of it remains as the neck of the polypide, and gives rise by cell proliferation to the body wall and the *Anlage* of a new bud. Thus the *Anlage* of each bud is part of that of a preceding bud. The question remains yet unsolved, Whence came the *Anlage* of the first polypide? Since the embryonic tissue of the inner layer of the bud, which seems to take the most active part in the formation of the bud, gives rise to both the lining of the alimentary tract and the wall of the brain, it becomes an exceedingly interesting question, From what germ layer is this inner bud layer derived?

In Cristatella, as in Plumatella, not all of the embryonic tissue from

which any bud arises goes to form that bud; but some of it is, apparently, passed along under the highly metamorphosed cells of the ectoderm, again to divide itself, one part going to form a new polypide, the other to form the *Anlagen* of new buds. In Cristatella, this embryonic mass of cells of the inner layer of the bud seems to be to a considerable extent independent of the highly metamorphosed ectoderm, and to form at places a sort of third layer, lying below the true ectoderm and above the muscularis with the cœlomic epithelium. Here, too, while it is easy to see buds arise from preceding buds in the adult colony, we cannot consider our question answered until we have discovered the origin of the cells from which, as from a stolon, the *Anlagen* of polypides successively arise.

I desire to say that I have avoided giving a full account of the ontogeny of these species, both because it is not directly required for the solution of the problems in hand, and because we are promised studies in this field by Braem.

The eggs of Phylactolæmata arise, as has long been affirmed, from the cœlomic epithelium of the body wall. The evidence of this is conclusive, for one often sees in a single section various stages in the development of the eggs. (Plate XI. Fig. 93, *ov'*.) It is also to be observed that they do not arise indiscriminately from any region of the body wall, but always close to the neck of a polypide. Sooner or later these eggs, surrounded it may be by a few follicular cells, are enclosed in an oœcium, and here undergo their development up to the stage of a young stock, possessing perhaps a dozen immature polypides. In the figures on Plates XI. and XII. the oœcium (*oœ.*) has been usually drawn, but in Figures 100 and 104 it has been omitted. As a result of cleavage, a blastula is formed, and from one pole of this — *the pole nearest to the neck of the oœcium* — cells are given off which move into the blastocœl (Figs. 94, 98) and finally come to line the cavity. It is important to observe that in the earliest stage of this process found there were four inner cells, of which two are represented in the section (Fig. 94, *ms'drm.* + *en'drm.*). Thus the two layers of the adult body wall are established. Up to this stage the conditions are practically the same in Cristatella and Plumatella. From now on, they are somewhat different in the two genera.

The first difference to be noticed is in the oœcium itself. In Cristatella the cells composing this rapidly become a pavement epithelium (Fig. 97); in Plumatella, on the contrary, the cells of the oœcium remain columnar (Fig. 99). The neck of the oœcium also differs in the

two cases. In Cristatella it is long, thick, and filled with a dense mass of large cells (Figs. 95, *cev. oœ.*, and 101 *, 102 *). In Plumatella (Fig. 99) it is very short.

The second difference concerns the embryo itself, and is connected with the formation of the first polypide. In Plumatella (Fig. 99) the first indication of the formation of the first polypide occurs at or very near the neck of the oœcium, or, since the ingression of cells into the blastocœl took place at the pole of the blastula nearest the neck, we may say near to the pole at which ingression occurred. The cells of the outer layer (*i.*) are elongated and contain large ellipsoidal nuclei which are often pressed close together. All of the cells of the larva stain more deeply at this pole than elsewhere, and those of the inner layer rather more deeply than those of the outer. The nuclei are also very large, those of the outer layer being possibly more prominent than those of the inner; but the difference is not so marked as in the drawing, where too the nucleoli of the inner layer are represented relatively too small. Even at this stage one finds in another section of the same embryo the beginning of a second polypide, whose position is indicated at *. This second polypide is indicated merely by a considerably thickened inner larval layer, and a very slightly thickened outer one. The two polypides are thus seen to be wholly independent of each other. The first invagination further advanced is seen in cross section of the whole larva in Figure 96. The entire outer layer would seem at first sight to be involved in this invagination; but even in this figure there are seen one or two nuclei which lie under the oœcium at the place of invagination. I believe that they will not be involved in it, for at a very little later stage (Fig. 104) one finds a layer of cells lying over the invaginated bud, which I believe are destined to form the ectoderm of the body wall at this place.

Later stages in the development of the larva in this species are not shown. The bud follows, I am confident, the same steps that are pursued by the bud in the adult colony. A placenta-like connection of the larva with the oœcium, which was first described by Korotneff ('87, p. 194), begins at about this stage, and continues until two well formed polypides are present. This "gürtelformige Placenta" begins to form in about the middle of the young embryo, and the elongated cell of the outer layer of the larva, in contact with the oœcium shown on the left of Figure 99 below the *, is, I believe, the first indication of it. The oœcium and larva both continue to increase in size, and the walls of the former become thinner with their increase in area.

The attachment of the œcium to the body wall of the mother stock always remains small, as in Figure 99, and the embryo, in my experience, does not come in contact with it.

The formation of the first polypide in *Cristatella* is preceded by another process. Just as in the adult colony the inner layer of the polypide does not arise by invagination of the ectoderm, but from the stolonic cells lying at the base of the ectoderm (see Davenport, '90, pp. 108, 109, Figs. 4 and 15), so too in the embryo. The first process then must be the formation of the stolonic cells. Figure 101 shows at the point marked *sto.* (which is at the pole of the embryo whence the inner-layer cells originated) that certain of the cells of the ectoderm appear to be arching over a disk, containing about six cells in section, and thus coming in contact with the cylinder of cells (*) which projects from the neck of the œcium. By a continuation of this process, the central disk of cells gradually comes to lie below the general level of the ectoderm, and to be cut off from contact with the neck of the œcium (Fig. 97, *sto.*). The position of the stolonic mass with reference to the neck of the polypide in this last figure must be considered abnormal; it is at any rate exceptional, as it lies at one side of the neck of the œcium, which does not, therefore, appear in this section. The next later stage which I have found is shown in Figure 102. The stolonic mass seen lying beneath the ectoderm in Figure 97 has here already given rise to a young polypide (*i., ex.*), and its area is increasing in all directions by cell division (*sto.*). The beginning of a second polypide is indicated on the right at *sto.* The ectoderm is seen lying above this stolonic mass, and closely applied to the neck of the œcium (*).

Neither at this nor at any subsequent stage have I been able to detect in Cristatella any "gürtelformige Placenta" such as exists in Plumatella. I am therefore of opinion that the process of nutrition, which is effected in Plumatella from the œcium through its placenta, is effected in the Cristatella larva by its attachment to the neck of the œcium. I am pleased to see that Jullien ('90, pp. 13, 14) has also reached this conclusion in a paper which he has had the kindness to send me. At a later stage, the embryo, or young colony, seems to become detached from its intimate association with the neck of the œcium, as we see in Figures 95 and 103.

Figure 103 represents a stage in which there are two well developed buds, both shown in the section. There is, in addition, on another section, one less developed. The stolon is seen passing oralward of these two

primary polypides, or rather the primary and secondary one. Moreover, as the series of sections shows, the stolon does not exist merely in this section, but it is a disk which is cut here in one of its diameters. A separation of the stolonic mass has occurred between the two oldest polypides, so that the ectoderm is here in contact with the cœlomic epithelium, just as is the case between buds in the adult stock. As the colony increases, the inner and outer margins of the stolonic tissue continue to extend farther outward, and this tissue forms at first a broad ring of ever increasing diameter. Later, as the area of the stock increases, the ring becomes broken, so that, instead of growing along an infinite number of radii, its growth is confined to a few, as in the adult colony.

I will defer a discussion of the significance of these facts to the general part of this paper.

B. GENERAL CONSIDERATIONS.

I. Laws of Budding.

Carefully conducted studies on stock building have generally revealed, just as these on Bryozoa have shown, a law in budding. This law in budding results in the formation of a stock the interrelation of whose individuals is a determinate one. I now propose to offer an hypothesis to account for the existence of these laws, and then to show how facts of budding in Bryozoa and other groups can be explained by means of it.

And first of all I must acknowledge that this hypothesis, although perhaps here first formulated, really depends upon observations and deductions made long ago on this group, first by Hatschek, who from 1877 has maintained that individuals do not arise independently of one another, and secondly and mostly to Braem, who in '88 (pp. 505, 506) declared of Phylactolæmata "dass in dem Stock keine Knospe entsteht, die nicht auf das embryonale, d. h. den specifischen Leistungen der Körperwand noch nicht angepasste Zellmaterial einer älteren Knospenanlage zurückgienge und dass somit in der ersten Knospe des keimenden Statoblasten sämmtliche Knospen des künftigen Stockes implicite enthalten sind." Not less is the following hypothesis indebted to the ideas of Roux and Fraisse, and to Nussbaum, who has said ('87, p. 293): "Ein lebendes Wesen ist somit als Ganzes oder in seinen Theilen soweit individualisirt und vergänglich, als die Gewebebildung und die Theilung der Arbeit vorgeschritten ist; das Ueberdauern der Einzelexis-

tenz, die Theilbarkeit auf geschlechtlichem oder ungeschlechtlichem Wege, spontan oder künstlich bedingt, ist an das Vorhandensein undifferenzirter Zellen gebunden und ist um so grösser, je weiter im Organismus diese Zellen verbreitet sind"; and, finally, to the idea which is implied in the conclusions of Nussbaum ('80, pp. 106–113) and Weismann, that germplasma does not find its origin in the parent individuals, but is merely borne by them in its unbroken passage from generation to generation.

This hypothesis is simply that *there is in every stock of Bryozoa a mass of indifferent cell material which is derived directly from indifferent cells of the larva or embryo, and whose function is to form the organs of the various individuals, including the polypides.* This indifferent cell material lies in the body wall, principally at the growing tip or margin of the stock. By its growth and differentiation it gives rise to the body wall, muscles, etc., and at intervals it leaves behind, as a portion detacned from itself, a mass of indifferent cells, which is capable of forming a polypide, or of becoming a new centre of growth, or of both. Which of these possibilities will be fulfilled, where and when these masses of indifferent cells will be left behind, depends upon the necessities of the species, and the variations in these respects give rise to the peculiar characters of the different stocks.

This hypothesis differs from that of Braem in that the pre-existence of a *Knospenanlage* assumed by Braem is, according to my view, a non-essential feature in the formation of the colony; the pre-existence of an indifferent cell mass, which does not itself constitute buds, but may give rise to masses which can, is the only essential feature.

As a first application of this hypothesis I refer the reader to the conditions of stock formation in *Paludicella*, already described. We find at the tip of the colony a mass of large proliferating cells, which I regard as histologically undifferentiated. These cells give rise to the body wall, — the cystid, — and at intervals leave behind three masses of cells, which I regard, from the fact that they retain their cuboid condition, as well as from their ultimate fate, as indifferent or embryonic. The median mass of each of these gives rise to a polypide, and to one only. The lateral masses form centres of growth similar to the one from which they were derived.

In order to reproduce the arrangement of individuals in the stock resulting from this manner of budding, we may make use of some graphic method of representation, as Smitt ('65[a], pp. 139, 140) did long ago, and as Allman ('70), Semper ('77, pp. 67–78), Chun ('88, pp. 1167–1180),

Braem ('90, pp. 33 and 44), Ehlers ('90, p. 9), and others, have since done. I shall represent the mass of indifferent cells by an asterisk, and individuals (according to Chun's nomenclature) by the use of the large and small letters of the Roman alphabet, and, finally, by Greek letters. The typical stock of *Paludicella* might then be graphically represented thus (cf. Plate I. Figs. 2 and 2ª) :—

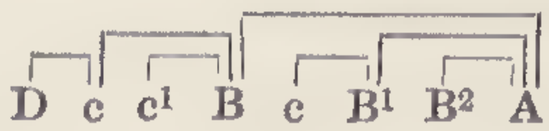
(1)

```
                                                    *
                                                  * c *
                                                  *   *
                                                * a b a *
                                                  *   *
                                                * *   * *
                                              * β a a a β *
                                                * *   * *

                                                    A

                                                * *   * *
                                              * β a a a β *
                                                * *   * *
                                                  *   *
                                                * a b a *
                                                  *   *
                                                * c *
                                                  *

                                    *
                                  * b *
                                  *   *
                                * a a a *
                                  *   *
                                    B
                                  *   *
                                * a a a *
                                  *   *
                                  * b *
                                    *

                        *
                      * a *
                      * C *
                      * a *
                        *

          *
        * D *
          *
```

Here the letters indicate polypides or their *Anlagen*, and the asterisks indifferent tissue. The individuals represented by capital letters may be called primary individuals; they may be said to belong to the primary series, and to have been derived from the primary indifferent mass. The individuals represented by small Roman letters will then be secondary individuals, belonging to the secondary series and arising from secondary masses, etc. It is to be observed that this indifferent tissue is here found only at the tips of branches or *Anlage* of such. No asterisks are found adjacent to the adult polypides A, B, C, etc., which have given rise to lateral branches, and these have therefore no power of producing new parts of the colony. The asterisks must not be regarded as having been descended from the letters which they adjoin, but from the terminal asterisks only; that is to say, in Paludicella embryonic tissue has originated from terminal embryonic tissue, and not from indifferent tissue left remaining alongside of the polypides.

Conditions differing in an interesting manner from these were found by Braem ('90, pp. 18–32) and myself (Davenport, '90, pp. 103–106) in Phylactolæmata. In *Plumatella* Braem has shown in the clearest manner how some of the embryonic tissue around a polypide at the proximal

end of a nascent branch is carried away to the oral side of the "mother polypide," and lays the foundations of another polypide. In like manner the embryonic tissue around the "mother polypide" may give rise to one or several additional embryonic masses. He has also (pp. 29–32) shown in the most convincing way that each mass, particularly in the case of secondary buds, consists of two parts, of which one goes to form the polypide; the other contributes to the further growth of the common cystid and the formation of new embryonic masses. Since here every embryonic mass is in intimate relation with a polypide, and since the polypides arise nearly in one plane, only secondarily moving out from it, the relation of individuals may be expressed by a formula occupying a single line. Braem has thus expressed it: —

(2) 

According to the system adopted for Paludicella, this may be given thus: —

(3) $*\alpha \; *a \; *b \; *A \; *a \; *B \; *C \; \text{✲}$

or, more developed, thus: —

(4) $*\alpha_1 \; *\alpha \; *\beta \; *a \; *\alpha \; *b \; *c \; *A \quad *\alpha \; *a \; *b \; *B \; *\alpha \; *C \; *D \; \text{✲}$

in both of which the right hand asterisk (✲) takes the place of the A at the right of Braem's diagram. These symbols denote that we have a mass of indifferent tissue connected with each polypide, or the *Anlage* of such; and this indifferent mass, as well as the adjacent polypide, was derived from some other indifferent mass. Thus the masses connected with A, B, C, D are to be regarded as having been cut off from the embryonic mass at the extreme right; and each of these secondarily gives rise to the polypide buds a, b, etc., and their embryonic tissue. Thus we have to do with centrifugal budding only.

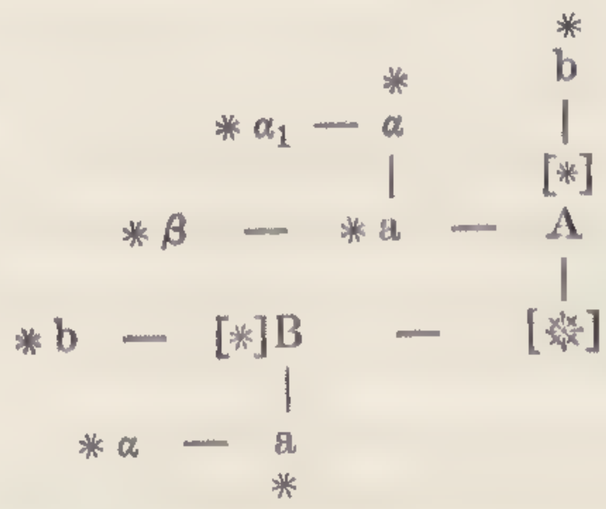
In *Cristatella* the conditions are essentially similar to those in Plumatella, the chief difference being that usually only two polypides with their embryonic masses arise from each polypide. This condition may be represented by the formula: —

(5) $*\alpha_1 \; *a \; *\beta \; *a \; *\alpha \; *b \; [*]A \; *\alpha \; *a \; *b \; [*]B \quad [\text{✲}]$

in which the embryonic masses originally attached to A, B, etc., are bracketed to indicate that they are normally no longer active in giving

rise to new polypides. As a matter of fact, the secondary rows often make a greater or less angle with the primary ones, and as a result lateral branches are formed. Taking this character into account, the Cristatella formula might be written: —

(6)



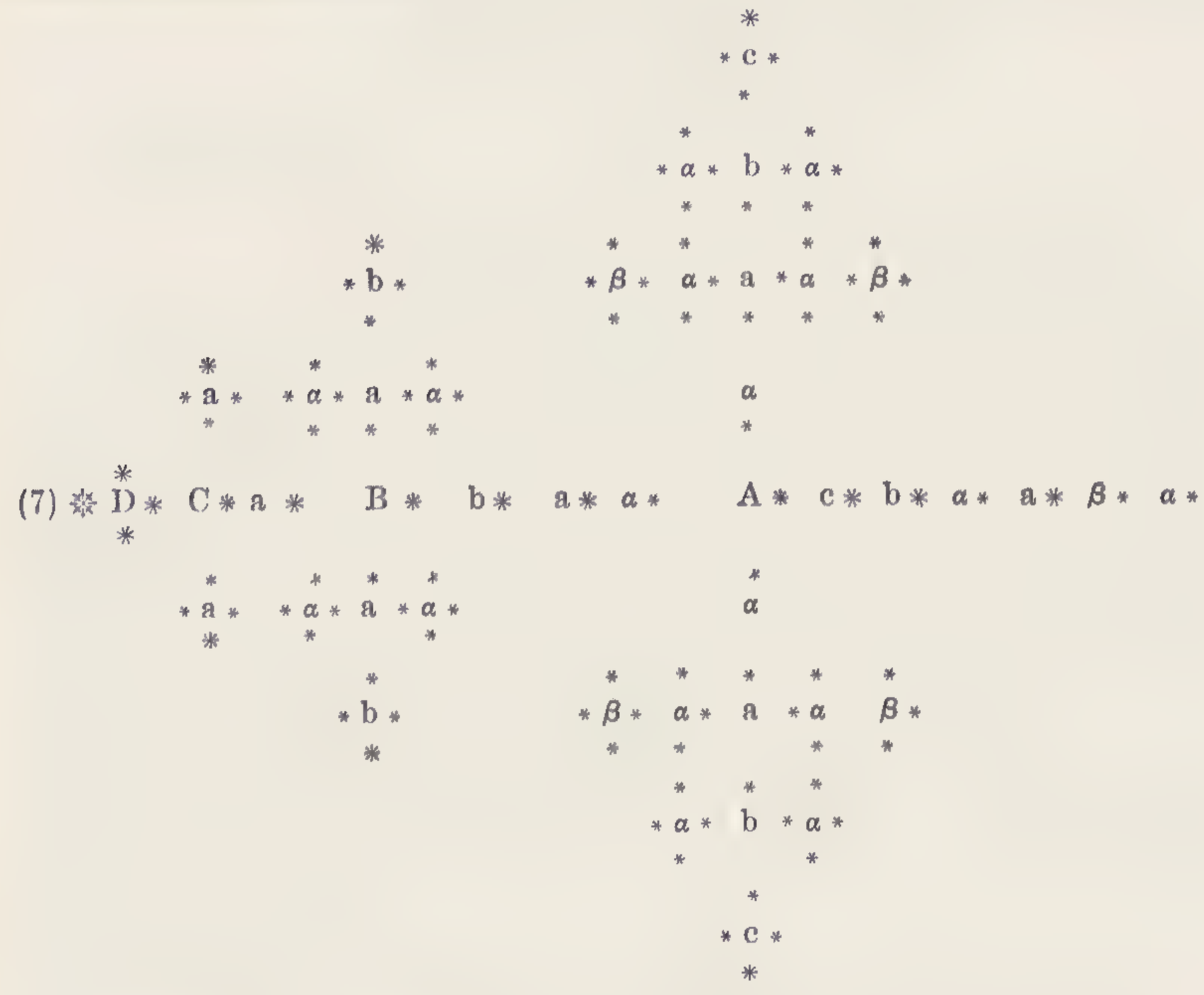
This representation indicates the fact that the first formed buds (A, a, α, etc.) are lateral ones; the second, median (Davenport, '90, p. 106). Intermediate stages between the condition in Plumatella, in which an indefinite number of polypides and gemmiparous masses can be budded off from pre-existing gemmiparous masses, and the condition in Cristatella, in which only two such arise, occur apparently in some species of Plumatella, in which, as Braem ('90, p. 31) has shown, few polypides are produced from any gemmiparous mass, and all but two of these generally do not develop. In the young corms of Cristatella, on the other hand, more than two polypides may thus arise.

Other *Ctenostomata* show a regularity in the budding process similar to that of Paludicella, and exhibit instructive variations upon it.

Victorella, an interesting Ctenostome occurring in slightly brackish water, and first described by Kent ('70) in 1870, possesses, according to the pregnant observations of Kraepelin ('87, pp. 75, 76, 154–157), a stolon-like tube, from which at intervals polypide-bearing "cylindrical cells" arise. Kraepelin ('87, pp. 155–159) has shown it to be in the highest degree probable that the protrusion of the body wall in the neck region of the polypide of Paludicella is the homologue of the "cylindrical cells" of Victorella, and that the remainder of the zoœcia of Paludicella is homologous with the "stolon" of Victorella. While in Victorella the cylindrical cell is developed to such an extent that the retracted polypide is still included within it, and the stolon remains of small calibre, in Paludicella, owing to its shortening, the retracted polypide must seek refuge in the stolon, whose diameter is consequently increased to receive it. Evidence for this is found in the stolon-like nature of the youngest zoœcia of a hatching winter bud of Paludicella Ehrenbergii, and in the elongated cylindrical cell of the adult Paludicella Mülleri, Kraepelin,

which must be considered a form intermediate between P. Ehrenbergii and Victorella.

The architecture of the Victorella and Paludicella stocks is, then, similar, in that they both consist of a row of individuals successively formed at a stolonic tip. The resemblance is heightened by the fact that, as in Paludicella, so also in Victorella, a pair of lateral buds is given off from each zoœcium to form lateral branches (Kraepelin, '87, p. 157). As in Paludicella, so also in Victorella, communication plates, *Rosettenplatten*, arise early to separate the zoœcia from each other. But Victorella differs from Paludicella in this, that while in the latter the neck of the polypide does not become the centre of origin of new buds, in the former it does, just as is the case in Plumatella (Kraepelin, '87, Plate III. Fig. 75) ; that is to say, there are laid down from the tip of the branch three masses of bud-producing tissue, besides that which goes to form the polypides of the primary branch. The graphic representation of this species will therefore be more complicated than that of Paludicella, and has this form : —

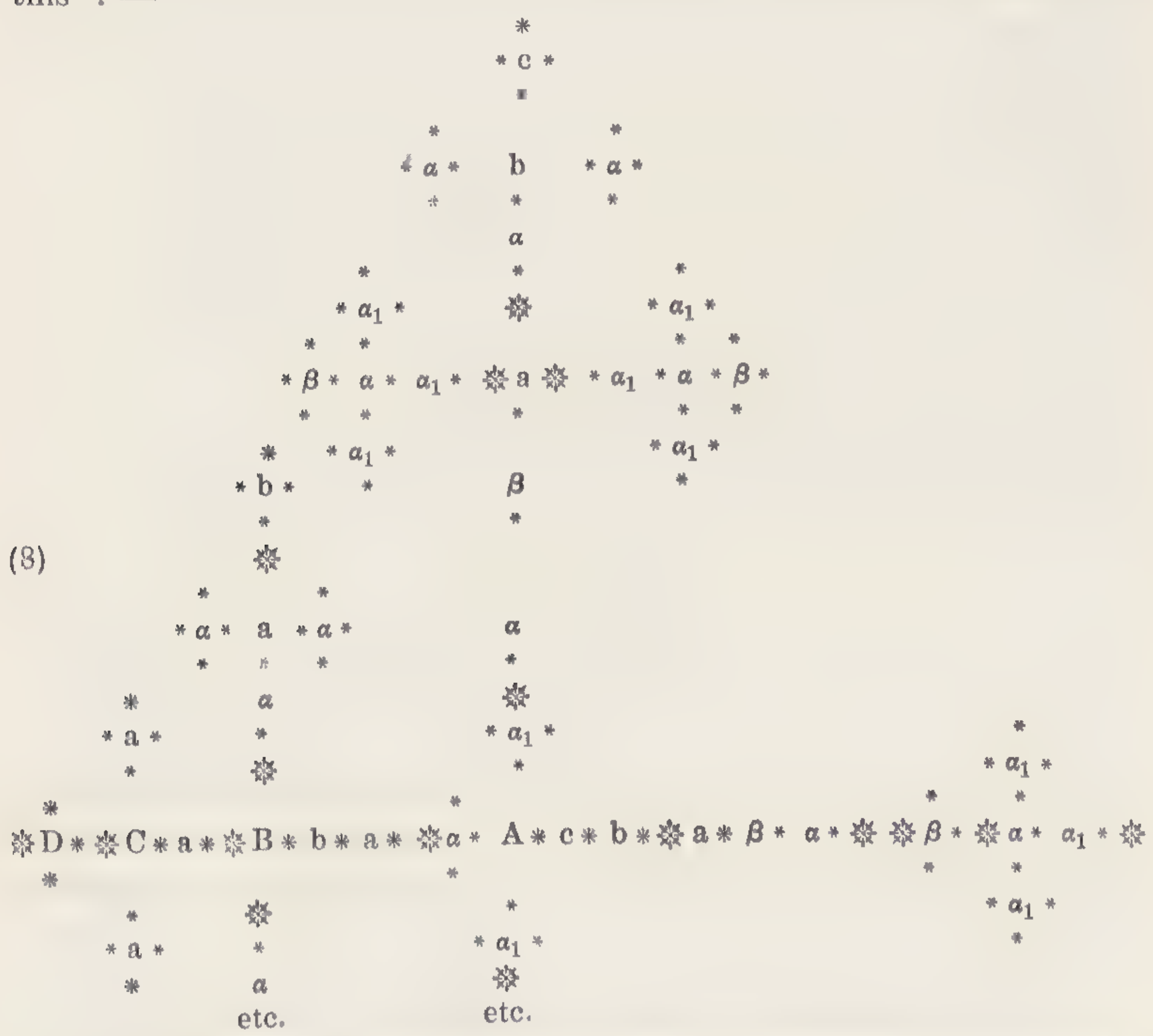


Compare with (1), page 73, and (4), page 74.

From around each individual of the series A, B, C, etc., which has been derived from the tissue of the stolon tip, there arise series of

lateral and of median buds. From around each of the lateral buds, in like manner, both lateral and median buds of a higher order arise. But from each of the median buds only median buds arise. These median buds, are not, however, all of the same kind. The one first produced (a *, of Formula 7, ✲ of Form. 8) differs from all formed after it (b *, c *, d *, etc.) in this, that it bears no polypide, but forms the tip of a stolon from which both median and lateral buds arise (β, α, near extreme right of Form. 8). From the second and all succeeding median buds (a b, c, etc., Form. 8), there arise only median buds of a still higher order. Of the latter, the first, as before, produces no polypide, but becomes the tip of a stolon giving rise to both median and lateral buds; the others give rise to only median buds of a still higher order, and so on. Our former formula assumed that all median buds were alike, and all incapable of giving rise to lateral individuals. Their dissimilarity introduces a complication, so that the species must be represented by some such formula as this [1]: —

(8)



[1] It must be borne in mind that such a graphic representation as this, while it agrees with the descriptions and figures of Kraepelin and Hincks ('80, Plate 79) so

in which the heavy asterisks represent the budding tips of the stock, which give rise to new individuals (tips of the stolons), and a_1, β_1, etc. indicate individuals of the fourth order. The lighter asterisks indicate, as before, points of proliferation from which new buds may arise.

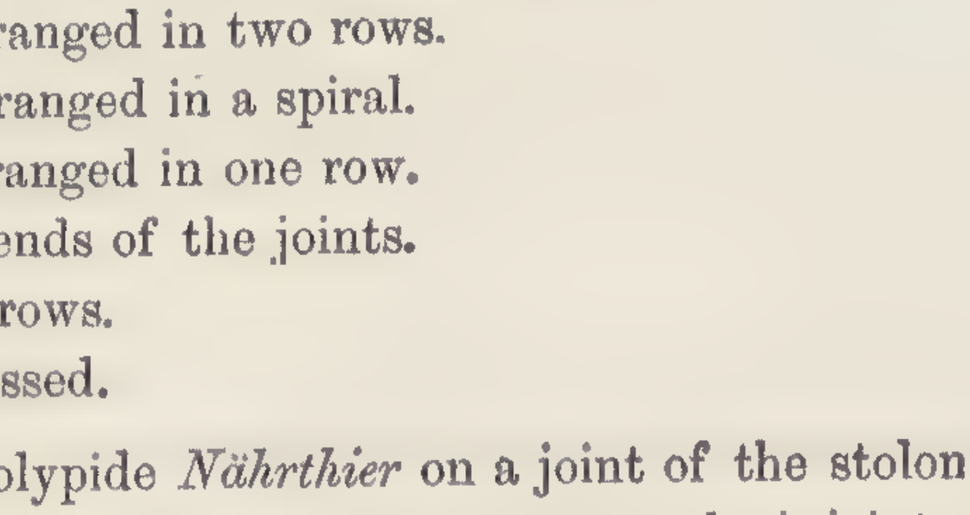
It seems highly probable that Victorella finds near allies in Mimosella and other genera of the Stolonifera.

In *Hypophorella expansa*, according to Ehlers ('76, pp. 5–9) and Joyeux-Laffuie ('88, pp. 137–139), the stolon is composed (as in Victorella), of a number of internodes, each separated from the other by communication plates, and bearing on the distal end typically a feeding zoöid (Nährthier) and a lateral stolon. It seems to me that the jointed condition of the stolon is reasonably accounted for in the same way as that of Victorella, by supposing that each internode, together with its zoœcium, is comparable with the whole indvidual of Paludicella. The "feeding zoöids" of Hypophorella will then be comparable with the *Cylinderzelle* of Victorella. Two facts are opposed to this view: first, the polypide is not formed primarily in the stolon, coming only secondarily to lie in the *Cylinderzelle* as in Victorella; and, secondly, there is a *Rosettenplatte* in Hypophorella between the feeding zoöid and the stolon, while none exists in Victorella. But upon this assumption one can best account for the fact that the stolon is composed of as many joints as there are feeding zoöids, — a condition which appears to occur in only a few other genera, and these closely allied to Victorella. Thus, in Cylindrœcium pusillum and C. dilatatum of Hincks we have two species which may be considered to represent two possible intermediate stages between Victorella and Hypophorella, not only on account of the jointed stolon, but also on account of the enlarged distal end of the joint, which is eminently characteristic of the allies of Victorella. The first objection, that the polypide is not developed in the stolon, but first arises in the well formed zoœcium of the feeding zoöid, might result from the increased importance of the zoœcium over the *Cylinderzelle*. The formation of the plate between the zoœcium and the stolon might be accounted for by the physiological need of such an organ resulting from the increased importance of the zoœcium (cf. p. 40). Such plates exist, in fact, between the primary median individuals, and those secondary median ones in Victorella which are budded from the *Cylinderzelle*. This hypothesis

far as they go, may not fit the conditions in all parts of the colony. Moreover, it is to a certain degree idealized, i. e. subjective, for even in the figure of Kraepelin ('87, Fig. 75) one of the individuals of the series a, b, c, etc. has given rise to no stolon as its first bud.

is further supported by the fact that, as a stolon may arise from the *Cylinderzelle* of Victorella, so in Hypophorella such a condition is not uncommon, although hardly typical. In accordance with this hypothesis the formula for Hypophorella might be given thus: —

(9)



Ehlers ('76, pp. 127, 128), in founding the group of Stolonifera, classified the different methods of arrangement of the individuals in the colony as follows: —

I. Many polypides (*Nährthiere*) on the single joints of the stolon (*Stengelgliedern*).
   1. On the entire length of the joints.
      (a.) Arranged in two rows.
      (b.) Arranged in a spiral.
      (c.) Arranged in one row.
   2. At the ends of the joints.
      (a.) In rows.
      (b.) Massed.

II. Only one polypide *Nährthier* on a joint of the stolon.
   1. Polypide lateral, near it one or many stolonic joints (Hypophorella).
   2. Polypide terminal.

In the present state of our knowledge, it is very difficult to say how the types of budding shown in those Stolonifera which possess more than one *Nährthier* on a joint of the stolon are related to, or are to be connected with, the types of Paludicella, Victorella, Hypophorella, or other genera possessing only one *Nährthier* to a joint. This could doubtless be determined, however, by studying the early stages in the development of the stocks. Taking them as they are, however, we find a very simple condition in the stocks of Class I., in which the *Nährthiere* are arranged in a single row, as in *Vesicularia spinosa* (cf. Hincks, '80, Plate 73, Figs. 3–7). The tip of the stolon consists, as I have myself observed in allied species, of somewhat cubical cells of variable thickness, and it is from this tip that the *Anlagen* of the individuals arise. Lateral branches occasion-

ally replace a *Nährthier*, and the latter seems never to produce secondary individuals. The formula of the stock might be written : —

(10)
```
                                                  *
                                                  b
                                                  a
  ✲ F — E — | — D — C — B — | — A
            a
            *
```

In *Bowerbankia pustulosa* we have two rows of individuals produced side by side from near the end of the stolon. This condition would be represented by

(11)
```
✲ * D  * C  * B  * A
✲ * D  * C  * B  * A
```

provided the individuals of this primary series possess the power of giving rise occasionally to secondary buds, as seems certainly to be the case in some members of this genus which I have seen. The spiral arrangement of some colonies is striking ; it is of evident advantage to the stock, but its cause in these cases is wholly unknown.

In every one of these cases, and, in fact, in all of those figured by Hincks, which belong to the Stolonifera, there is no trace of dichotomy. Throughout we have to do with linear series, which give rise to lateral branches.

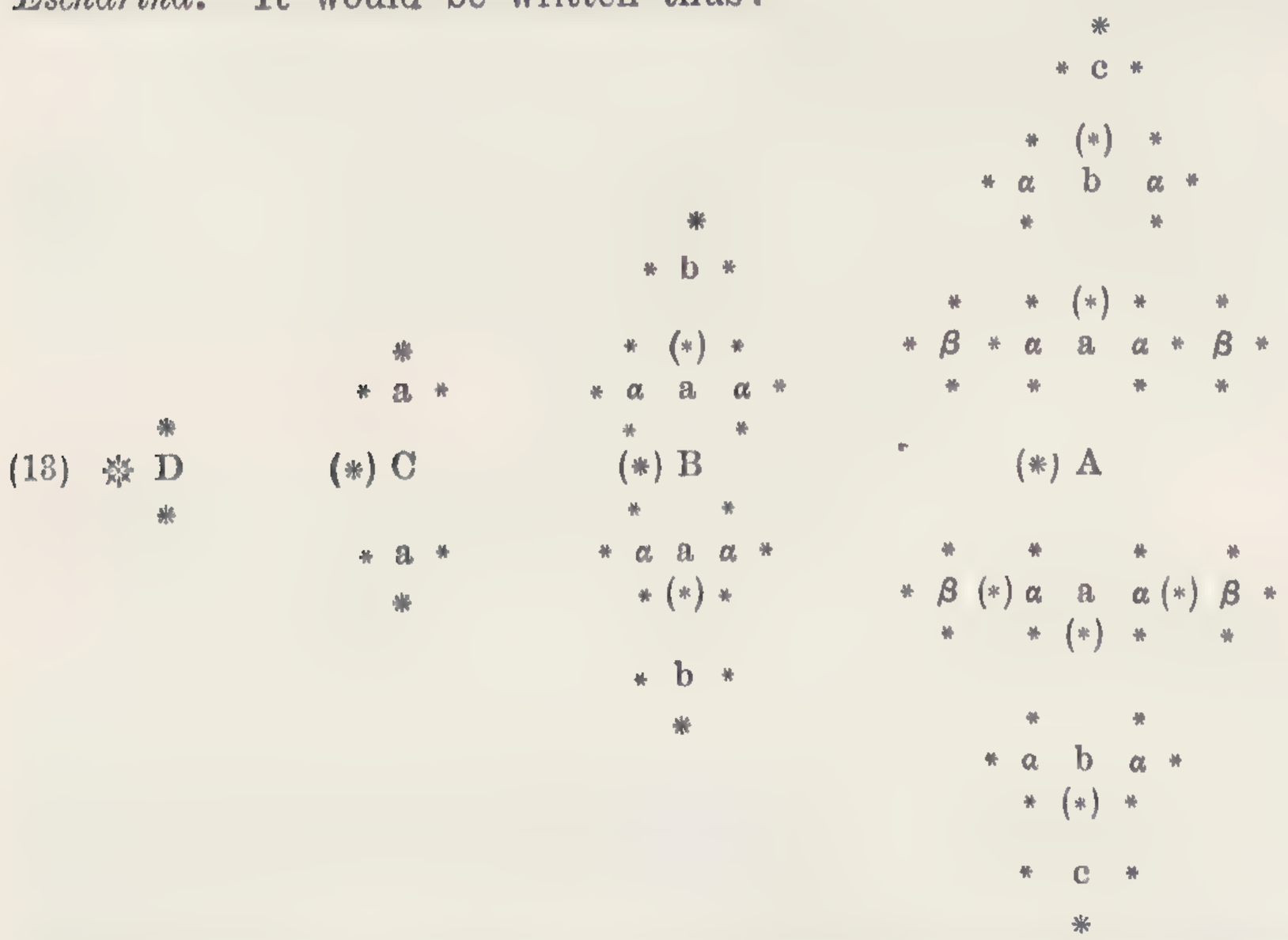
Turning now from the Stolonifera to the other grand division of Ctenostomata, the *Alcyònidæ*, we find the same prevalence of a law in budding. In its typical expression it may be written as follows : —

(12)
```
                                  *
                                * b *
                    *         * (*) *
                  * a *     * a a a *
          *                   *   *
        ✲ C       (*) B     (*) A
          *                   *   *
                  * a *     * a a a *
                    *         * (*) *
                                * b *
                                  *
```

Although secondary median individuals are not habitually formed, yet, owing to the capacity of regeneration possessed by individuals A, B, C, etc., an asterisk is affixed in parentheses to show the probable persistence of embryonic tissue. Of the lateral series one or both may fail to be developed.

It might be difficult to determine whether in this group we have to do with dichotomy, did not the tips of the margins at times reveal the fact that there is no division of the ancestral series, but that a new one is added at the side of an ancestral one (Plate VIII. Fig. 69), where of the marginal individuals 4 is clearly median (ancestral) and 3 is lateral, 13 median and 12 lateral, etc. (see page 49).

The members of the group of *Cyclostomata* seem to be closely related, and the method of budding is so similar throughout the group that it seems fair to interpret the more compact Tubuliporidæ from the Crisiadæ. In *Crisia*, as we have seen, individuals are placed in rows, from which at intervals lateral rows are given off to the right or left. One may say that typically these are given off from each individual to both the right and the left, although in some cases, as in Figure 65[a], lateral branches are typically given off alternately to the right and left, and are often aborted. Perhaps the most general formula of all for Cyclostomes should be that of two lateral branches from each individual, one or both of which may remain undeveloped. Such a formula I believe to be also the typical one for *Bugula* and its allies, and for the *Flustrina* and *Escharina*. It would be written thus: —



in which the parenthesized asterisks indicate the presence of regenerative tissue. This is identical with (12) and similar to (1).

Braem ('90, pp. 130–133) has already called attention to the difference between Phylactolæmata and Gymnolæmata in the *orientation of the*

polypide. In Phylactolæmata the oral aspect of the polypide is turned towards the margin of the corm or the tip of the branching stock; in Gymnolæmata, on the contrary, the anal aspect is turned in that direction. This difference is a very striking and constant one. It is correlated with another difference in the law of budding of the stock, which will become evident upon comparing Formulas (4) and (5) on page 74, of Phylactolæmata, with Formulas (1) on page 73 and (7) to (13). In all of these the margin or tip of the stock is at the left, the centre at the right. In the formulæ of Phylactolæmata the budding is *centrifugal*, new individuals being produced from the embryonic masses towards the margin; in the formulæ of Gymnolæmata budding is *centripetal*, new individuals being produced from the embryonic masses towards the centre. *In both Phylactolæmata and Gymnolæmata the anal aspect is turned towards the gemmiferous region.*

Braem calls attention to one other difference, namely, that, in the case of the retracted polypide, in Paludicella the rectum lies next the attached surface of the stock; in Phylactolæmata, the œsophagus. A mechanical cause of this is suggested when this statement is put in other words: the polypide in its retracted position is stored in both Phylactolæmata and Gymnolæmata proximad of the atrial opening; i. e. away from the tip or margin, and towards the centre of the stock. May not this be explained, in part at least, as an adaptation to room?

I will here add four examples of regular budding taken from other groups of animals, to illustrate the general applicability of this method of representation. The first of these is that of the Siphonophore *Halistemma* whose formula has been worked out by Chun ('88, p. 1169), and expanded and illustrated by Korschelt und Heider in their recent textbook (p. 39). It runs as follows: —

(14) D c b a C d c b a B e d c b α a γ β α A

According to my interpretation of the case, this formula might be written (15): —

✲D ✲c ✲b ✲a ✲C ✲d ✲c ✲b ✲a ✲B ✲ ✲e ✲d ✲c ✲b ✲α ✲a ✲γ ✲β ✲α ✲A,

in which the ✲ behind B has been derived from the embryonic mass at A, that behind C from B., etc. The ✲'s represent embryonic masses from which a, b, c, etc. are derived.

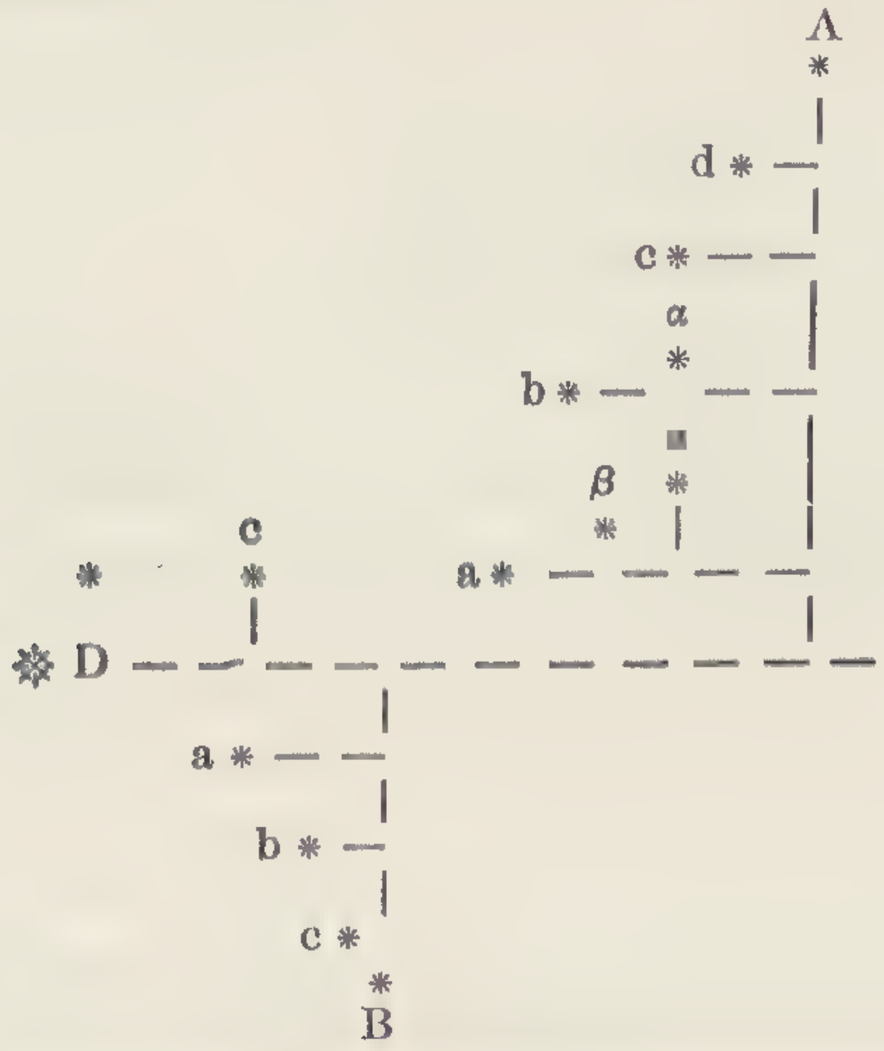
If we assume that the terminal individual (A) has not been derived from the primary embryonic mass, at the extreme left, but has had its

origin in the embryo, the formula would have to be written somewhat differently ; namely, thus (16) : —

(16) ✵ C✵ c* b* a* B✵ d* c* b* a* A✵ e* d* c* b* α* a* γ* β* α* [A]

In a species of *Pennaria*, common on our coast, which is probably Pennaria tiarella, McCready,[1] I have noticed the presence of a similar law of budding. The whole stock lies in one plane, the lateral branches arising alternately from the right and left of a central stock, like the barbs of a feather. These lateral branches give rise to a series of secondary ones, which are all placed on the same (axial) side of the branch. Each branch, of whatever degree, originates as a bud bearing a polyp. From the elongating stalk of this terminal polyp, buds arise, — the beginnings of branches of the next higher order. The stock may be represented by the following formula : —

(17)



Expressed in a linear series, this formula may be written : —

(18) ✵ D* C* B* c* b* a* A* d* c* b* α* a* β* α*

which is identical in form with the second formula (16) given for Halistemma.

[1] This species is figured by Leidy ('55, Plate 10, Figs. 1–5) and Verrill ('73, Plate XXXVII. Fig. 277). An allied species, P. gibbosa, is figured by Louis Agassiz in the "Contributions" (Vol. III., Plate XV. Fig. 1). In describing P. Carolinii, Weismann (Entstehung der Sexualzellen, p. 122) says that the lateral hydranths do not possess the capacity of giving rise to new lateral hydranth-buds (of a higher order). But, as indicated above, P. tiarella seems to do this regularly. Leidy's and Verrill's figures show the same thing.

Lastly, this formula may be applied to certain cases of fission, as in *fresh water Annelids.* As is well known, the fissiparous process is preceded by the formation of the so-called budding zones (Knospungszone). These arise in *Ctenodrilus*, according to Kennel ('82, pp. 403, 404), between two dissepiments in the middle of a metamere, and new ones are continually formed behind the others as the animal grows in length by cell proliferation at the tail end. The budding zones are, according to Kennel, regions composed of embryonic cells. I think it probable that this embryonic tissue has been derived from the embryonic tissue of the anal end of the animal. There are as many budding zones produced as there are new metameres added by the anal growth, and since the budding zones are intrasegmental, each zoöid consists of four parts; viz. (naming them from anterior to posterior end) of the posterior half of the preceding budding zone, of the posterior half of the metamere in which the budding zone arose, of the anterior part of the next following metamere, and, finally, of the anterior part of the following budding zone. Zoöids then are made up of parts of two adjacent metameres, and the middle of each zoöid is intersegmental. The zoöid has progressed little beyond the state of possessing two (half) metameres at the time it becomes free. New metameres must become formed by caudal growth. The animal is, then, according to my conception of the significance of the process, derived chiefly from these budding zones. Evidently, the law of production of new individuals (or new budding zones) is a simple one, and may be written, in accordance with my nomenclature,

(19) ⁂ E (*) * D (*) * C (*) * B (*) * A

in which A, B, C, etc. represent successive individuals (adjacent halves of two metameres), and the asterisks, as before, embryonic tissue. The two adjacent asterisks together represent the budding zone, of which the posterior half (parenthesized) proves itself the least active.

The conditions given by Semper ('77, pp. 69, 77) for *Chætogaster* (and Nais) are much more complicated, but may be expressed by the use of a formula constructed upon the same plan. Chætogaster differs from Ctenodrilus in this: that young budding zones, and eventually young individuals, are produced between older ones, instead of always at the anal end; and the new zoöids often acquire several metameres before becoming free. It seems to me probable that, as in Ctenodrilus, the budding zones are derived ultimately from the anal zone; but here, in contradistinction to Ctenodrilus, new budding zones may secondarily arise from other budding zones produced earlier, thus

giving rise to the phenomena of young individuals interpolated between older ones. Representing, then, individuals by the budding zones from which they have arisen, we may convert the following formula of Semper into one based on our own nomenclature :

$$\overset{\text{E}}{\underset{\sim}{3}}+0-0\quad\overset{\text{D}}{\underset{\sim}{4}}+0-\overset{\text{H}}{\underset{\sim}{1}}+0-0\quad\overset{\text{B}}{\underset{\sim}{4}}+\overset{\text{G}}{\underset{\sim}{1}}+0-0\quad\overset{\text{C}}{\underset{\sim}{4}}+0-\overset{\text{F}}{\underset{\sim}{2}}+0-0\quad\overset{\text{A}}{\underset{\sim}{4}}+1$$

az.

in which the succession of generations of zoöids is

... 5, 4, 8, 2, 7, 3, 6, 1.

In the above formula A, B, C, etc. represent zoöids; the numerals below the letters, the number of metameres of which each is composed; 0, an incomplete metamere about to be derived from a budding zone; *az.*, the anal zone. Written in accordance with my conception of the facts, this formula would read :

(20) ⁂ D (*) * C (*) * a (*) * B (*) * b (*) * a (*) * a′ (*) * A,

which somewhat resembles Formula (15) of Halistemma, and signifies that two embryonic masses are left behind by the anal zone, of which the one anterior to the zoöids proper (represented by letters) goes merely to form the head parts, and is represented parenthesized. The second is caudad the zoöid, and may form a secondary "anal zone" giving rise to new zoöids. From one zoöid two or more anal zones may take their origin. Thus, from the embryonic mass caudad of A there have arisen that caudad of b, which has given rise to b (*) * a (*), and that caudad of a′, which has given rise to a′ (*).[1]

The most general formula given on page 81 undergoes many modifications in the different groups, but in the midst of these modifications certain laws are to be discerned, to some of which I have already called attention. I will now proceed to a discussion of the significance of these laws.

The *quincunx* arrangement of individuals, which is so noticeable in

[1] From a study of surface views of many specimens of Autolytus collected at Newport during June, 1891, I am convinced that the sexual individuals are produced by proliferation of cells in the metamere XIII. or XIV. of "parent form," — the last which remains behind after breaking off of sexual form. Representing the proliferating metamere by (⁂), we may write the budding formula of Autolytus thus:

(20ª) ⁂ E (*) D (*) C (*) B (*) A (*)

in which the parenthesized asterisks indicate the proliferating, but not gemmiferating anal metameres of the sexual form. (Cf. A. Agassiz, '63, pp 397–400.)

the phytoid stocks of Bugula, and in creeping corms like Lepralia or Cristatella, may be explained as affording additional strength on the one hand, and as a device for saving space on the other.

The absence of true dichotomy, which I have sought to show characterizes the budding of Bryozoa, is interesting as seeming to indicate the fundamental similarity of the process of budding in Paludicella to that found elsewhere. The tip of the branch does not divide equally in the first nor in the other instances, but constantly maintains its precedence, giving off parts of itself to form lateral branches. These parts may grow out at right angles to the primary branch, as in Paludicella, but generally they grow forward nearly parallel to it, as in most marine Gymnolæmata.

In Bugula (Plate VII. Fig. 64[a]) branches are always given off *toward the axils*, and therefore an ancestral branch gives off all lateral branches from one side and the successive orders of branches are given off alternately to the right and left. In Crisia, on the contrary, branches are given off *abaxially*, and they are given off not from one side only, but alternately to the right and left. In both cases the two facts are mutually dependent. The first case gives rise to a stock in which the branching tends as greatly as possible towards compactness and the formation of a closely built stock; the second case gives rise to a diffuse and loosely built stock (cf. Figs. 64, 65, and 64[a], 65[a]). In the second case there is a maximum space to each individual; in the first, a maximum *economy* of space.

The rule that lateral buds on two closely related branches tend to arise in the same generation is one that, as has been shown, is more or less apparent in some cases, but is easily obscured by other rules. May not the tendency be due to the same causes that produce the synchronism of division in related cells of a cleaving egg?

That lateral buds should occur in *Bugula flabellata* on the outermost rows only is not surprising when we reflect that there is abundant room on the margin, whereas the inner individuals are hemmed in from lateral expansion by the pressure of adjacent rows. This is very marked in certain repent colonies, as, for instance, occasionally in Membranipora (Plate VIII. Fig. 70). Here the intermediate branches 6, 7, 8, and 9 have produced no lateral buds for many generations, while almost every individual of the marginal rows has given rise to a lateral branch. It is merely a result of the same cause, it seems to me, that lateral budding occurs more frequently in *Bugula turrita* at the margins of fans than elsewhere. Here there is room to spread.

The rule (5) that ancestral rows contain fewer *generations* of individuals than lateral ones may perhaps receive a partial explanation from the further fact (rule 6) that of the two rows starting from any axil the ancestral branch will give rise to a greater *total number* of individuals than the lateral one will in the same time. We should expect a less rapid forward growth if the lateral growth is extremely vigorous. One might also say that the intermediate rows had grown abnormally in length, since that is the direction in which there is most room.

The reason why the ancestral branches in Bugula give rise to the greater total number of individuals is, to my mind, because they are marginal. In Crisia it is the lateral branches which are the most prolific, and for the same reason.

The existence of the 7th rule in mat-like species is a mechanical necessity; in the phytoid species, like Bugula and Crisia, it must be accounted for on another ground; namely, on the relations of food supply to demand, — on the deterrent effects of overcrowding. And this, to my mind, is the key to the significance of the 4th, 5th, 6th, and 7th rules. The form of the stock is determined by the same law which has determined the form of the individuals, — the struggle for existence and the survival of the fittest, — the fittest in the present case being those which are most advantageously placed with reference to food supply. Abundant food supply has made possible the rapid production of lateral individuals at the margin, and less abundant food supply has retarded such production in the middle. Therefore has lateral budding occurred more rapidly at the margin; therefore has the number of individuals produced at the margin been greatest; therefore have the median rows grown *in length only* with great rapidity; therefore has the distance between adjacent rows of individuals in phytoid stocks remained constant.

Many observations on different groups of animals agree in demonstrating a relation between rapidity of the budding or fission process and food supply. Thus Zoja ('90, pp. 25–27) has shown for Hydra, and Zacharias ('86, p. 274) and von Wagner ('90, p. 360) for Turbellarians, that abundant food supply results in an acceleration of the processes of non-sexual reproduction, and Braem ('90, p. 24) has shown that budding in Cristatella proceeds less actively during the late fall. This diminution in activity has been attributed by Braem to diminished temperature; but we know also that this period is one of scarcity of the small fresh water organisms upon which the fresh water Bryozoa live (cf. Parker, '90, pp. 597–600), and this fact also must be considered as having an important influence in this case.

II. Relation of the Observations on Budding in Bryozoa to the Germ Layer Theory.

No question in Bryozoan morphology has been more thoroughly discussed than that of the part played by the germ layers in the production of the polypide, and upon none has there been less agreement. Nitsche first boldly opened the question, and concluded that we have in this process a fatal objection to the idea of the homology of the germ layers, in so far as their homology depends upon a similarity of fate throughout the Metazoa. A single layer, the invaginated ectoderm, gives rise to the outer covering of the tentacles, to the pharynx, and to the brain, — structures elsewhere considered as ectodermal, — and also to the lining of the alimentary tract, elsewhere universally accounted entodermal. In view of these facts, "sind die Keimblätter," concludes Nitsche ('75, p. 398), "keineswegs mit einer besonderen histologischen Prädisposition ausgestattete Zellschichten, sondern lediglich die flächenhaft ausgebreiteten Elemente, aus denen die den Metazoenkörper zusammensetzenden, ineinander geschachtelten Röhren sich bilden." Prouho, although recognizing the facts to be as stated by Nitsche, has not discussed the theoretical bearing of the question. Seeliger ('89[a], p. 204) finds in the budding process of Endoprocta a shortening and confusion of the embryonic process. "Wie die gesammte Knospenentwicklung verkürzt ist, erscheinen auch die beiden Processe der Einstülpung durch welche im Embryo zuerst Entodermkanal, dann Atrium sich bilden, in einen zusammengezogen." In another place (Seeliger, '90, p. 595) the budding process is considered as an "immer sich erneuerende Gastrulationsvorgang." Braem ('90, p. 116) regards the inner layer of the bud as entoderm, and the process of its formation as one of gastrulation. In a preliminary notice published last February (Davenport, '91, p. 279) I suggested that the embryonic tissue from which the inner layer of the polypide arises is to be regarded as "neither ectoderm nor entoderm, but as still indifferent, and capable of giving rise to either." A few weeks ago I saw for the first time the paper of Oka ('90), in which he offers (p. 145) *a priori* a similar suggestion concerning the significance of the embryonic tissue from which the inner layer of the polypides arises. I am pleased to find that our ideas, thus independently arrived at, are so fully in agreement. My idea of the relation of the germ layers to the layers of the polypide bud chiefly grew out of my studies on the embryology of Phylactolæmata as described in earlier pages.

As there are two layers to the bud, the question of the part taken by

the germ layers in the polypide bud may be subdivided into two: What is the significance of the outer layer of the bud? and, What is the significance of the inner?

The *outer layer of the bud* is derived from the cœlomic epithelium. The views of those who have studied the formation of this inner layer of the cystid in Phylactolæmata may be classed in two categories: (1) those in which it is regarded as entoderm, and the process of its formation as gastrulation; and (2) those in which it is regarded as mesoderm. To the former class belong the views of Reinhard ('80, p. 208), Korotneff ('89, p. 403), and Jullien ('90, p. 19); to the latter, those of Kraepelin ('86, p. 601) and Braem ('90, p. 116), and in this class the views of Barrois ('86, p. 68) and Haddon ('83, p. 543), founded on *a priori* considerations, must be placed.

It seems to me that, since, as Barrois has demonstrated, there is a great similarity between the Phylactolæmatous and Gymnolæmatous larvæ, and especially since the former show evident signs of degeneration, we are bound to study the phenomena they exhibit in the light of our knowledge of the ontogeny of Gymnolæmata.

But first it is necessary to give reasons for believing that the larva of Phylactolæmata is to be regarded as homologous with that of Gymnolæmata; and to do this I will first name the points of similarity in the two larvæ, and then try to show that the differences which exist are not sufficient to invalidate the attempt to establish a homology. And, first of all, it may be said that, since the adult Phylactolæmata and Gymnolæmata are strikingly similar to each other, and since no one doubts their close relationship, we should expect *a priori* that their larvæ would be homologous, especially since the larvæ of Gymnolæmata are admitted to belong to the trochosphere type, of whose ancient origin there can be little doubt. In the second place, the very existence of a larval stage in Phylactolæmata is indicative of its inheritance from an earlier condition, for two reasons: (*a*) because in general fresh-water life tends to eliminate larval stages from species which have inherited them from marine ancestors, and tends little to form them *de novo* (Hydra, fresh-water Turbellarians, Rotifera, Oligochæta, Hirudinea, Astacus, and fresh-water Mollusca); and (*b*) because, specifically, the early stages of development of Phylactolæmata are passed within a uterus-like sac, from which the embryo is released only when a colony is already well established. In the third place, the Phylactolæmatous larva possesses, in common with all Gymnolæmatous larvæ, the following characteristics. The primary polypides arise in both at a pole, and this pole is in both a prominent disk,

surrounded by a circular fold, — the so-called mantle fold, — from which it is separated by a circular groove, — the so-called mantle cavity. This organ has a similar origin and fate in the two groups, as shown by Barrois.

The following points of difference, however, must be recognized. First, the absence of a definite ciliated ring, *couronne* (Barrois), of an internal sac, and of a pyriform organ. But, as Barrois ('86, p. 67) has shown, these are absent, or at least (Ostroumoff, '87, pp. 182, 183) little developed, in Cyclostomatous Bryozoa. The ciliated ring and pyriform organ are doubtless organs connected with a free locomotive larval life, which is greatly abbreviated in Phylactolæmata. A second difference exists in the fact that, while most Gymnolæmatous larvæ possess either, rarely, (1) a functional alimentary tract, or (2) a mass of loose tissue lying inside of the ectoderm, the Phylactolæmata possess (3) a central space lined by an epithelium placed next to the ectoderm. However great the difference between the first and third conditions mentioned above, it is to a large extent bridged over by the widespread existence of the second. In some Cyclostomes, moreover, a similar condition to that in Phylactolæmata seems to exist. Compare Metschnikoff ('82, p. 310, Taf. XX. Fig. 62). Lastly, the origin of two primary polypides, instead of one, at the aboral pole, upon which Barrois has laid some stress, cannot be considered a very strong objection to the homology, because in reality the two polypides do not arise at the same time even in Plumatella, and in Cristatella this difference is still more pronounced. In fact, it is not the formation of *two polypides* which requires explanation, but that of a young *stock* before hatching.

There remains, therefore, to my mind, no serious objection to regarding the larvæ of Phylactolæmata and Gymnolæmata as having been derived from some common ancestral larva, possessing, of course, more points of resemblance to the Gymnolæmatous than to the Phylactolæmatous type; and therefore it is perfectly justifiable to interpret the latter by aid of the former.

Admitting the larvæ to be homologous, we should expect the process of *gastrulation* to be comparable throughout Ectoprocta. As a matter of fact, we do find a great similarity in the earliest stages. Thus, the first indication of the inner layer is the ingression of four cells at one pole, which by multiplication give rise to a layer of cells lying inside of the ectoderm.[1] It is to the comparative study of the fate of this inner

[1] This has been shown for Membranipora (Tendra) by Repiachoff ('78, pp. 416–420); for Alcyonidium polyoum by Harmer ('87, pp. 445, 446); for Bugula by Vigelius ('86, p. 519); and for Cristatella in the present paper (page 68).

layer in the different Ectoproct larvæ that we must look for an explanation of the layer in the specific case of the Phylactolæmata.

For the purposes of this study, it is desirable to begin with species in which there has been a minimum amount of degeneration. Such are Membranipora (Cyphonautes), Alcyonidium, and Flustrella, to which we must now turn our attention.

The studies of Repiachoff on Membranipora lead up to a stage in which the entoderm lies as a solid mass inside the ectoderm, and is separated from it at all points. Neither the origin of the mesoderm nor the formation of stomodæum or proctodæum was observed at this time. As for the fully formed Cyphonautes, it is certain, as I can confirm from personal observation, that there is a well developed functional alimentary tract, and that it is provided with a well developed muscular system, including cross-striped muscle fibres. There is, therefore, every reason for believing that typical entoderm and mesoderm have been formed in it.

In Alcyonidium (polyoum), Harmer ('87, p. 445) has shown that after gastrulation a great mass of cells occupies the former blastocœl. This, in the author's opinion, represents entoderm and mesoderm. The young larva possesses a mouth, an œsophagus, and a large stomach, but never an anus. No evidence is presented that the oral pole corresponds with the pole of ingression.

Flustrella, which is nearly related to the last species, possesses in its young larval stages a pocket, which Prouho ('90, pp. 424–426) has shown to represent the anterior part of the alimentary tract, directly comparable with that of Alcyonidium polyoum, but less developed. Mus cle fibres and an epithelial lining of the entoderm and ectoderm exist to indicate the presence of mesodermal tissue.

These three genera, Membranipora, Alcyonidium, and Flustrella, are the only Ectoprocta in whose larvæ the presence of an alimentary tract has as yet been demonstrated.

In Bugula, a very careful study of which was made by Vigelius ('86 and '88), one finds after gastrulation and cell multiplication a mass of cells filling the whole interior of the larval body, at first appearing as an epithelium surrounding a central space, but later without arrangement and often showing signs of degenerescence. No definite separate mesoderm could be found, and at no time was any trace of an alimentary tract to be seen. Vigelius calls the mass derived from the four entodermal cells *Füllgewebe*, and he believes it to correspond morphologically to both "hypoblast and mesoblast." It is to be noted, however, as a point of considerable importance, that in his figures of the metamorphos-

ing larva Vigelius ('88, Taf. XIX. Fig. 6) represents this tissue as having almost entirely disappeared; that which remains giving rise to the mesodermal lining — the outer layer of the bud — of the developing polypide.

There can be no doubt that the so-called oral pole of the Bugula larva corresponds to the mouth-bearing pole of Alcyonidium, but does it correspond to the pole of ingression of entoderm? This question has not been answered by Vigelius. The existence of homopolar stages like that represented in his ('86) Figure 25, Taf. XXVI., makes it very difficult to establish this doubtful point.

The formation of the inner layer of Cyclostomes has been studied by Barrois ('82, p. 141). He says: "Dès les premiers stades les sphères vitellines glissent les unes sur les autres de manière à former une espèce de gastrula par épibolie et l'on ne tarde pas à rencontrer des stades d'un volume extrêmement exigu et déjà composés d'une couche exodermique et d'une masse endodermique libre dans son intérieur. La masse endodermique s'atrophie rapidement et l'on arrive à une petite blastula qui succède non pas à un stade composé de cellules radiaires dans lequel se forme une cavité centrale, mais qui est issu, au contraire, d'une vraie gastrula née par épibolie dans les premiers stades de la segmentation et dans laquelle la masse endodermique est déjà disparue." I have quoted Barrois thus at length, since his description will show forcibly at least one thing, that the fate of the cells which by ingression had entered the blastocœl is quite different from that of those in Bugula, where a great *Füllgewebe* is formed. Ostroumoff ('87, p. 183), however, has shown that the inner layer of the Cyclostome larva does not disappear, but comes to line the ectoderm as a very thin layer. In the adult larva, however, we find the contents of the ectodermal sac "filled with mesenchymatous cells, which are commingled with yolk granules and globules of albumen." It is these cells that produce the very considerable mesodermal layer of the first polypide, which arises in the metamorphosis of the larva. Here, as elsewhere, an apparently homopolar stage intervenes between gastrulation and the formation of larval organs, making orientation difficult.

Thus, passing from Cyphonautes, through Alcyonidium and Flustrella, Bugula, and finally Cyclostomes, we have a series in all of which the inner germ layer is derived from one pole by ingression or by 'epiboly," and in which there is a gradual reduction of the functional entoderm until it seems, in Cyclostomes, to be lost, and a gradual transformation of the mesoderm from a cell mass nearly filling the larva, and producing muscles

and a lining to the body wall and alimentary tract, to a single thin cell layer lying next to the ectoderm, or to mesenchymatous cells extending through the cœlom.

This same series may be said, also, to be one in which there is a gradual decline in the complexity of larval organs. These find their maximum development in the bivalve Cyphonautes and Flustrella, and the complicated and beautiful Alcyonidium larva. They find their minimum development in the Cyclostomes, whose larvæ, instead of a girdle of flagella, possess merely an undifferentiated clothing of cilia, are reduced to a cylindrical or ellipsoidal form, lack the pyriform organ of other species, and in some cases possess only the rudiment of the internal sac.

If we were to imagine still another term at the degraded end of the series, it would be a form in which the four inner-layer cells that arise by ingression at one pole of the larva should give rise to little or absolutely no entoderm, in which the mesoderm should come to form an inner lining to the ectoderm, and in which the internal sac should be entirely absent. It is just these conditions which are fulfilled by the Phylactolæmatous larva.

Of all these changes, the loss of the entoderm is the most striking. What can be said in explanation of it? I would suggest this hypothesis: that *the entoderm of the Bryozoan larva has become rudimentary through loss of the alimentary function.*

In direct support of this hypothesis I have little experimental evidence to offer. One observation, however, which I made last summer, seems to favor this conclusion strongly. This is that larval life is of considerable duration in Cyphonautes, which possesses a functional alimentary tract, but is very brief in Bugula, in which no alimentary tract arises. As is well known, Cyphonautes occurs in enormous numbers in the "tow" at certain seasons of the year, and this is alone evidence of a considerable length of life. I have taken Cyphonautes thus obtained from the tow and have kept them for three or four days, at the end of which time they died, or had settled to the bottom of the glass vessel to undergo their metamorphosis. In fact, from several hundred Cyphonautes which I collected, not more than half a dozen completed their full metamorphosis, the others apparently succumbing to unfavorable conditions.[1]

[1] Just as the manuscript of this paper is going to the printer, after long delay caused by an accident necessitating the re-engraving of the plates, I find that Dr. Prouho read last summer ('90), before the Association Française pour l'Avancement de la Science, a preliminary communication on the development of Cyphonautes. This is published in the printed report of the proceedings of that association. The

The *Bugula* larvæ, on the contrary, I have never found in the tow, but they swarm out from stocks gathered in the morning and placed in a glass vessel; and I can confirm Nitsche's ('69, p. 9) observation that they settle and begin their metamorphosis within "a few hours" after hatching. One rarely or never finds these larvæ succumbing to the unfavorable conditions of the aquarium before metamorphosing. From these observations I conclude that the Bugula larva has a very much shorter life than Cyphonautes. Now, since the larva, owing to its shortened life, has no need of functional entoderm, and since entoderm can be of use to the larva only, no part of it going over into the tissues of the primary polypide of the stock (except as food material), functional entoderm is not developed. In other genera, its rudiments have become less and less important in the ontogeny, and, finally, in Phylactolæmata are wholly lost.

That the entoderm should reach its last stage of degeneration in *Phylactolæmata* is easily understood when we consider that the larval period is passed in a closed oœcium, from the wall or neck of which it receives nourishment *as a parasite does*. Moreover, by the delay in the period of hatching, as well as by precocious development of polypides, one at least of the latter is usually functional in the just hatched stock, for there is sometimes found at least one polypide in the newly hatched larva, which is partly extruded, and therefore capable of feeding, and thus of supplying the whole stock with nutriment. Of what advantage to a species could be the development of a functional larval entoderm, which should go to form no part of its adult tissue, provided the larva was contained in a uterus during its early stages, and was provided with the adult digestive organs in a functional condition before leaving the uterus?

Those who maintain that the inner layer is to be regarded as entoderm, and are still unwilling to place the Bryozoa among the Cœlenterata, must account for the absence of mesoderm. Korotneff ('89, p. 400) finds degenerating cells in the blastocœl before this is wholly obliterated by the extension of the inner layer. These he seems to regard as degenerate mesoderm. According to his view, then, the entoderm gives rise to the muscularis, — for this arises from the inner larval layer, according

author does not there state whether stomodæum and proctodæum are formed on the blastoporic side of the larva. He accounts for the existence of an alimentary tract in Cyphonautes by the fact that it undergoes its development disconnected with the parent, while almost all other Bryozoa pass their early stages in the parent or some protecting zoöid (oœcium, ovisac, ovicell).

to Braem's ('90, Taf. VII. Fig. 89 *mb.*) observations, which I can abundantly confirm, — and to the cœlomic epithelium of the adult stock. In the few series of sections of the proper stage which I possess, I have not found with certainty the degenerating cells of which Korotneff speaks; but even if they regularly occur, I should be inclined to regard them as the degenerated entoderm, the mesoderm persisting to give rise to the muscular tissue and the cœlomic epithelium.

From a consideration of these facts, — that the larvæ are homologous and the process of gastrulation is comparable throughout the Ectoprocta, that in the least modified larvæ both functional entoderm and mesoderm are produced by that gastrulation, that one of these two germ layers has become rudimentary in Phylactolæmata, that it is highly probable that the entoderm has disappeared from loss of function, and that the layer which persists gives rise to the musculature, sexual cells, and cœlomic epithelium, — I conclude that *the inner layer of the Phylactolæmatous larva, and therefore the outer layer of the bud, is mesoderm.*

If we accept the point of view of Kleinenberg ('86, pp. 1–19) and admit the existence in general of only two layers, ectoderm and entoderm, a clearer conception of the modification undergone by the Phylactolæmatous larva may be gained. We may divide the entoderm arising in Bryozoa into two parts; viz. (1) that which gives rise to the lining of the midgut, as in Cyphonautes, and (2) all the rest of the inner layer. Now, since no midgut is formed in the Phylactolæmatous larva, part (1) of the entoderm has ceased to be differentiated; all which remains, then, is part (2); but this is equivalent to "mesoderm" in the sense in which I have employed it, and therefore I am justified in saying that "mesoderm" only is produced.

The question has now to be answered, What is the significance of the *inner layer of the bud?* Two different answers have been given to this question. It has been maintained, on the one hand, that it is to be regarded as ectoderm; on the other, as entoderm. There are serious difficulties in the way of accepting the first view, — so serious, in fact, that few authors have maintained it, although at first glance it seems to be required by the facts. Although we have not yet sufficient grounds for declaring that organs formed by budding must be built up from the same germ layers as corresponding larval ones, — although we may admit that gemmigenesis recapitulates phylogeny and corresponds with ontogeny only in an imperfect and confused way, — still, from the experience gained by tracing the development of hundreds of animals from

the most widely separated groups of the animal kingdom, the idea that a functional alimentary tract is ever wholly derived from differentiated ectoderm will not be accepted by most embryologists without conclusive evidence.

The second view is that the formation of the inner layer of the bud is a process of gastrulation, giving rise to entoderm, and that the so-called "gastrulation" of the sexual ontogeny of Phylactolæmata is to be regarded as a precocious ingression of mesoderm only.

Two considerations are opposed to this view. In Membranipora there is a gastrulation which gives rise to the entoderm and mesoderm of the larva; and since the gastrulation of Phylactolæmata is similar, these elements must be potentially present here also. The "gastrulation" in Bryozoa is a normal one; if there is any entoderm in the body wall giving rise to the inner layer of the bud, it must have been entoderm which failed to become invaginated. But what, in the second place, is to be gained by assuming that the inner layer of the bud is formed from entoderm? Here is as great a difficulty as before, since the nervous system originates from this layer. It has been maintained in many cases that the nervous system arises from *mesoderm*, and Seeliger ('89, p. 602) believes that it is formed from that layer in the non-sexual reproduction of some Tunicates; but I know of no good evidence of its origin in any of the Triploblastica from *entoderm*.

Before going on to state my conception of the significance of the inner polypide layer, I desire to call attention to the conditions in the region at which it is first formed. I have shown above (page 69) that the primary polypide or polypides arise from the pole of ingression in Phylactolæmata, and that therefore in this group the aboral pole (in the sense of Barrois) corresponds to the pole of ingression. As I understand Barrois, he means by oral pole merely the pole which in Cyphonautes, for instance, bears the mouth, — the pole also by which the larva attaches itself. Braem ('90, p. 123, foot-note), however, interprets "oral side" in Barrois's sense to mean in the last instance the place at which gastrulation takes place. Perhaps Barrois does somewhere state such to be the significance of his term (I have not found the place), but in that case I can only say that, to my mind, he has not produced sufficient evidence to prove that the oral pole of the larva of Gymnolæmata is the same as the pole of ingression in the gastrula; nor, in my opinion, has any other investigator done so. Nearly all species studied have a stage early in their development when their poles are very similar, and orientation certainly would be exceedingly difficult. One of the

best figured series in which to trace the homology of poles is that shown by Repiachoff ('80, Taf III.) for Bowerbankia. So far as the figures go, one would conclude that Figure 10 A and its predecessors were oriented in the opposite direction to Figure 11 and its successors, which would result in placing the pole of ingression (Fig. 9) at the aboral pole of the larva, — the pole which here, as in all other Gymnolæmata, and, I believe, in Phylactolæmata also, gives rise to the primary polypide. I have given above additional evidence for this conclusion, in my argument to prove the homology of the larvæ and larval organs in Phylactolæmata and Gymnolæmata.

The polypides arise in Phylactolæmata at the pole of ingression, which is probably homologous with the aboral pole of Gymnolæmata. The pole of ingression, or the region of the lips of the blastopore, must be regarded as being a region of less pronounced differentiation than the rest of the gastrula. Its cells cannot be said to be either ectodermal or entodermal. It is an interesting fact, that it is just these indifferent cells — not yet either ectoderm or entoderm — that give rise to the inner layer of the polypide, from which organs usually considered ectodermal as well as those considered entodermal arise.

My conclusion, then, the objections to which I fully realize, may be stated in the following words: *The inner layer of the polypide bud is composed of cells derived from the rim of the blastopore. Such cells are to be regarded as still indifferent, and as first becoming differentiated into ectoderm and entoderm in the formation of the young polypide.*

Just when and where, on this hypothesis, the differentiation into ectoderm and entoderm occurs, is an important question; but unfortunately I cannot answer it decisively. It may be pointed out, however, that it has now been shown for most Ectoprocta that the lining of the middle part of the alimentary tract is formed independently of the œsophagus, and by an actual or potential outpocketing of the primitive simple sac of the bud. In Endoprocta there is a similar outpocketing, which, however, arises in connection with the œsophagus, and is formed independently of the rectum.

This is perhaps the proper place to call attention to the fact that the mesodermal outer layer of the bud has a very embryonic character at the budding region. This is indicated by the fact, that in Phylactolæmata (in which group alone I have studied the subject) eggs always arise from that part of the cœlomic epithelium which lies in the budding region (cf. Plate XI. Fig. 93). In Pyrosoma, also, according to the researches of Seeliger ('89, pp. 598–602) the mesoderm of the budding

region, the stolon, gives rise to eggs. The same condition seems to exist in other Tunicates.

III. On some Characteristics of Gemmiparous Tissue.

In the preceding part of this paper the words "embryonic tissue," "undifferentiated tissue," have often recurred, and they are terms in wide usage in modern zoölogy. I do not know of any attempt to define further the real character of this tissue, nor to give its more detailed characteristics, other than that usually employed in the term *plasma-reich*, or "rich in plasma." The persistence of yolk granules is, as Nussbaum ('80, pp. 2–14) and Goette ('75, pp. 31, 32, 831) have shown in the case of amphibian embryos, indicative of the *embryonic* condition of cells, when these have been derived from an egg filled with yolk.

It is very far from my purpose to go into a detailed discussion of the significance of embryonic tissue, for which I am not yet fitted; nevertheless, I wish to call attention to the minuter characters of gemmiparous tissue as I have found it in Phylactolæmata and Paludicella. I have described it in some detail in preceding pages.

First, then, gemmiparous tissue seems to stain more deeply than non-gemmiparous tissue in the same section. This character has been repeatedly observed before by others, and Braem calls attention to it several times. I have already described how I found, by the use of high powers, that much of this depth of stain was due to the unusually large number of deeply staining granules scattered through the cell, but chiefly gathered about the nucleus (Figs. 6, 17, 18, etc.). So marked is the greater depth of the stain around the nuclei, that, with a power so low that the nuclei are hardly distinguishable, their position is indicated by a deeply staining band.

Secondly, gemmiparous tissue, as I have found it in the cases referred to, is distinguished by the possession of large cells, nuclei, and nucleoli. I had already noticed this fact in my studies on budding in Cristatella, and I find that Braem has figured the nuclei in the budding region as larger than the average (cf. Braem, '90, Taf. VII. Figs. 86, 88–90). My own figures show this repeatedly (Plate I. Figs. 3, 4, 5, 6, Plate II. Figs. 15, 17, Plate XI. Fig. 99, etc.). I have also noticed this to a certain extent in the marine Bryozoa, but, since the cells of the latter are smaller, and as I did not succeed in obtaining from them sections so satisfactorily stained, the results are not so reliable. In attempting to obtain an explanation of this phenomenon one involuntarily recalls to

mind the condition in young egg cells, where the nucleus attains a relatively enormous size. This great size of the nucleus in young egg cells is explained by Korschelt ('89, p. 92) as due to its participation in the trophic activity of the cell: "Sein grösster Umfang fällt in die Zeit des energischen Wachsthums der Eizelle." So in the gemmiparous regions the large size of the nuclei must be considered as connected with the growth of the cells.

But if the growth of the cells is accompanied by a rapid ingestion of food material (which the larger nucleus implies), some evidence of that fact should be observed in the cells themselves in the presence of food granules. Such food material in rapidly growing ovarian egg cells lies near the nucleus. Stuhlmann ('87, pp. 13, 14) describes such a condition in the ovary of Zoarces. "Neben dem Keimbläschen, jedoch ein klein wenig von seiner Membran entfernt, bilden sich an verschiedenen Stellen jetzt eigentümliche Verdichtungen des Protoplasmas, die sich ein wenig stärker mit Saffranin färben als das Zellplasma." Such a thickening of the protoplasma is represented in the figures as minute granules. Korschelt ('89, pp. 123–125) mentions several other such instances.

It has seemed to me possible to interpret the stainable granules lying near to the nucleus in gemmiparous tissue as such food material,[1] particularly since we know that food material dôes exist in the cœlomic epithelium lying next to the cells which are about to divide rapidly and to give rise to the inner layer of the polypide. That food is being taken in by the inner layer cells from the cœlomic epithelium is indicated by the fact that the nuclei of the former cells lie near the latter epithelium (cf. Figs. 15, 17, 18, 28, 56, etc.); for, as Korschelt has shown, the nucleus tends to move towards the centre of activity of the cell. That these

[1] Granules similar to these appear to exist in the protoplasm of all cells. It is their extraordinary abundance in the gemmiparous tissue upon which I lay stress. They have been variously interpreted by different authors. Bütschli ('88, pp. 1469–1472) describes various kinds of stainable granules in Ciliata which are food products, and the general character of which accords with that of the granules referred to above. "Excretion granules" of Ciliata do not stain, according to this author, which is an indication that the bodies in gemmiparous tissue are not such. I am particularly struck by the fact that the food products of Protozoa are chiefly found in parasitic forms, — Gregarinidæ and parasitic Ciliata. These take up food in solution from their hosts exactly as the cells of the body wall of Bryozoa do from the body cavity. Altmann ('90) has recently interpreted similar deeply staining granules in other cells, as "die Elementarorganismen." I can see no reason, on Altmann's theory, for the peculiar distribution of the granules that I have found.

granules observed in the cells are food material is indicated by their abundance in cells lying next to the reticulated cells of the cœlomic epithelium (Figs. 6, 28, 56).

My conclusion, then, is this: *Gemmiparous tissue is a rapidly assimilating tissue, possessing large nuclei because actively assimilating, and staining deeply because full of food material.*[1]

While for Nussbaum, as already quoted (page 71), "indifferent cells" are essential to the reproduction of individuals by non-sexual as well as by sexual methods, Seeliger ('90, p. 596) has concluded that "die Vorgänge bei der Knospung der Bryozoen uns zeigen, wie histologisch sehr bestimmt differenzirte Gewebe einen ganz embryonalen Charakter wiedergewinnen können. Mehr noch als bei der normalen Knospung am freien Stockende ist dieses Vermögen bei der Regeneration der Polypide der Ektoprokten oder der Köpfchen der Pedicellinen ausgebildet. In diesen Fällen sehen wir ein plasmaarmes, äusserest feines Plattenepithel, das über sich eine mächtige Cuticula ausgeschieden hat, sich in kubische und cylindrische plasmareiche Zellen zurückverwandeln und durch eine Einstülpung ein neues Polypid bilden, in welchem schliesslich die mannigfachsten Gewebsformen vertreten sind."

It seems to me that many facts in the budding of Bryozoa are strongly in favor of Nussbaum's hypothesis. On this assumption, we can best understand why in Cristatella there is not an invagination of the ectoderm, and why instead a stolon is formed in the embryo, which passes along at the base of the ectoderm and at intervals gives rise to the inner layer of the body wall. I believe it is because the outer layer of the body becomes so rapidly differentiated by the secretion of the

[1] Other observers describe gemmiparous tissue as being either rich in food or deeply staining. Seeliger ('85, p. 588) speaks thus of the mesodermal gemmiparous tissue in Salpa: "Die einzelnen Zellen sind grossblasig, enthalten einen runden Kern und führen Oel- und Fettsubstanzen die als Reservematerial beim Aufbau des embryonalen Leibes weiterhin in Verwendung gelangen." Von Wagner ('90, p. 377) says of the indifferent cells which are being transformed into the new pharynx of dividing Microstoma: "Dieselben nehmen an Grösse zu, . . . indem gleichzeitig ihre Protoplasmaleiber feinkörnig granulirt und für Farbstoffe imbibitionsfähiger werden."

In some sections of gemmules of Esperella fibrexilis, H. V. Wilson, of which Dr. Wilson has very kindly sent me several slides, I find the outer layer of young gemmules, in which the inner layer has been newly formed, stained very deeply. Observed with a Zeiss Apochr. 4.0 mm., Ocs. 8 and 12, the cell contents are seen to be evidently of two kinds, — light and deeply stained. The latter appearance is due, in part at least, to small dark granules, which can be discerned without much difficulty.

gelatinous balls in its cells as to be incapacitated for the work of building organs. In Plumatella the outer layer of the body wall, which is derived, as Braem has shown, from the neck of the older polypide, retains for a long time its embryonic condition, so that its deeper cells can and do go to form the inner layer of the polypide bud.

On Nussbaum's hypothesis we can best understand why in Paludicella the *Anlagen* of the lateral branches exist from the beginning as cuboidal cells, quite different from those of the rest of the body wall; we can understand why the cell layers of the margins of the stock, the tips of branches, and the ends of stolons from which buds arise, are thicker and more rapidly dividing than the rest of the body wall (cf. Figs. 14, 71, 73, 75); and we can also understand why the regenerating buds always arise from the region of the neck of the degenerated polypide, — the same region from which that degenerate polypide had arisen by budding.

There is no doubt, however, that at times buds do arise from tissue which, as Seeliger says, has lost its cuboidal nature only to regain it. From such tissue apparently the polypide of Figure 79 has arisen; from such tissue certainly, as Seeliger says, do regenerating polypides arise. But is the process by which cuboidal cells become a pavement epithelium one of so fundamental differentiation that, in accordance with Nussbaum's doctrine, we should not expect, under favorable conditions, to see these cells regain their cuboidal form? No doubt we have many other cases in the animal kingdom in which flat epithelial cells regain their cuboidal form. Thus, for instance, among the Bryozoa, Oka ('90, p. 132) has shown how the flat cells of the outer layer of the statoblast begin to thicken again at the return of warmth, and at the beginning of the active assimilative processes, not only at the pole from which the primary polypide is to arise, but also opposite to this.

Many facts indicate that cells may become flattened epithelia, and yet not lose their embryonic character. Maas ('90, pp. 541–544) has recently shown step by step how the columnar ectoderm of the fresh water sponge is forced, on account of the great increase in surface which it is called upon quickly to cover, to become broad and flat. It finally gives rise to an epithelium so flat that its existence was long overlooked, and has been denied by so competent an observer as Goette; and yet in its flattened condition it possesses to a remarkable degree the capacity of sending out pseudopodia-like processes, a condition indicative much less of a high degree of differentiation or specialization, than of an unspecialized, primitive or embryonic condition.

I have already stated (page 65) that the region from which the regenerating buds of Cheilostomes arise, although one of flattened epithelium, is one in which many more nuclei persist than elsewhere in the adult (cf. again Fig. 71). This fact, coupled with the constancy of position of regenerating buds with reference to the degenerated polypide, is to my mind evidence against the assertion that buds arise here from "*histologically very definitely differentiated tissue.*"

As for regeneration in Endoprocta, no one is more competent to speak than Seeliger himself. I am the more surprised, therefore, to find that in Ascopodaria macropus, which is quite closely allied to the species studied by Seeliger ('89), the cells at the part of the stalk immediately below the "head," from which regenerated buds arise, are, as Ehlers's magnificent Pedicellina work shows, very large and cuboidal (Ehlers, '90, Taf. II. Figs. 26–33). I think one may conclude that a similar condition obtains in some cases in Pedicellina, even judging from Seeliger's own drawings, although they are drawn to a scale that is not quite large enough to allow of settling this point (Seeliger, '89, Taf. X. Fig. 35, *a*, Fig. 41, etc.).

If the increase in size of the flattened cells, and their subsequent rapid division and invagination to form a bud, are due to their more active nourishment, it would be difficult to see why certain cells of any region should quickly undergo this modification, while the adjacent cells apparently as favorably situated with reference to the acquirement of food retain their flattened, quiescent condition, if we assumed such favorable situation to be the only requisite. Still less satisfactorily would such an assumption explain the regular position of regenerating buds. It is taking only one step farther back, but, to my mind, a helpful step, to assert that cell proliferation in any region which produces invagination depends upon the capacity of the cells of that region to become better nourished than their fellows. This may evidently be effected by a diminution in the feeding capacity of the surrounding cells, or by an increase in this respect in the growing cells.

IV. Relationships of Endoprocta and Ectoprocta.

I discussed this topic in my earlier paper (Davenport, '90, pp. 132, 133). I have only to add, that later studies have confirmed my opinion of Nitsche's correctness in placing these two groups close together, and in regarding the Endoprocta as nearer the ancestral types. The stages of Figures 25 (Plate III.) and 77 (Plate IX.) probably rep-

resent roughly a phylogenetic stage ancestral to both groups of Bryozoa, but most clearly allied to adult Endoprocta. The formation of new tentacles anteriorly and posteriorly in Figure 77, would reproduce the adult Endoproct condition. Two changes lead to the Ectoproct stage: first, the closure of the tentacular corona posteriorly *in front* of the anus (Plate V. Fig. 43), and, secondly, the formation of the pharynx or anterior part of the œsophagus by the growth of the oral tentacles over the floor of the atrium towards the atrial opening. Thus the brain, which lies at the floor of the atrium in Ectoprocta, comes to lie on the pharynx. The pharynx would, upon this assumption, be a new structure, not found in Endoprocta. Such appearances as are exhibited by Figure 77 lead me to retract my former expressed opinion, in which I agreed with Nitsche in saying that the earliest condition of the tentacular corona is a U-shaped one. Rather, the tentacles are formed first on each side of the atrium, and only secondarily grow around the mouth in front, as later they grow in between mouth and anus. The U-shaped stage is therefore not the primary one, but secondary.

The close relationship of Endoprocta and Ectoprocta has recently been doubted by Cori ('90, p. 16), but his chief argument depends upon the dissimilarity of the Endoproct and Ectoproct kidney. Unfortunately, our knowledge of the latter is still very imperfect, and we may well hope for renewed researches in the subject by this skilful investigator.

Ehlers ('90, pp. 149–154) has recently re-expressed his former ('76, p. 132) utterances concerning the lack of homology between the tentacles of Ectoprocta and the "cirri" of Endoprocta. He finds the homologue of the latter in the "Diaphragma" or "Kragen" of Ectoprocta. This is the organ which I have believed to be homologous with Kraepelin's "Randwulst" (which may be Anglicized as marginal thickening), — an organ occurring in all Ectoprocta. It is nothing but the "neck of the polypide," which has sunk below the general level of the body wall. It is always provided with sphincter muscles, and in Ctenostomes forms the base of insertion of the cylindrical or comb-like "collare setosum." It can hardly be that Ehlers refers to this latter structure by the term "Kragen," since this is merely cuticular. In my opinion the "Diaphragma" of Nitsche cannot be homologized with the cirri of Endoprocta, because it is merely a part of the body wall comparable to that part from which the "polypide" of Endoproctous Bryozoa arises, and *beneath* which the tentacles or "cirri" arise. This part of the body wall is provided with a sphincter in Endoprocta as well as Ectoprocta, and by it the atrial cavity may in both cases be closed.

To my mind, the most significant difference between the two groups exists in the fact that the outpocketing to form the stomach arises from the oral end of the future alimentary tract in Endoprocta, and from the anal end in Ectoprocta. One is led to believe that in the ancestral form either two nearly equally important outpocketings from both the oral and anal sides existed, or that the two existing methods are remnants of a method different from either (such as the formation of the whole alimentary tract at once), or, finally, that the Endoproct condition represents the ancestral one, and that the rectal evagination has secondarily become of greater importance in Ectoprocta, and that the oral evagination has become less significant. Oka ('90, pp. 134, 141) has recently asserted that in the polypide buds of the statoblast and adult colony of a Pectinatella of Japan (P. gelatinosa) the œsophagus and stomach are formed by one evagination, which acquires secondary connection with the rectum. This condition reminds one, then, of Endoprocta. I must, however, doubt the accuracy of Oka's conclusions until more satisfactory evidence is forthcoming; the more so, since Pectinatella magnifica, Leidy, presents a method of budding exactly comparable to that in Cristatella and Plumatella, as my own sections show with sufficient clearness.

The homology of the Ectoprocta and Endoprocta implies a homology of their larvæ, and demands that the life history of the two groups should be directly comparable.

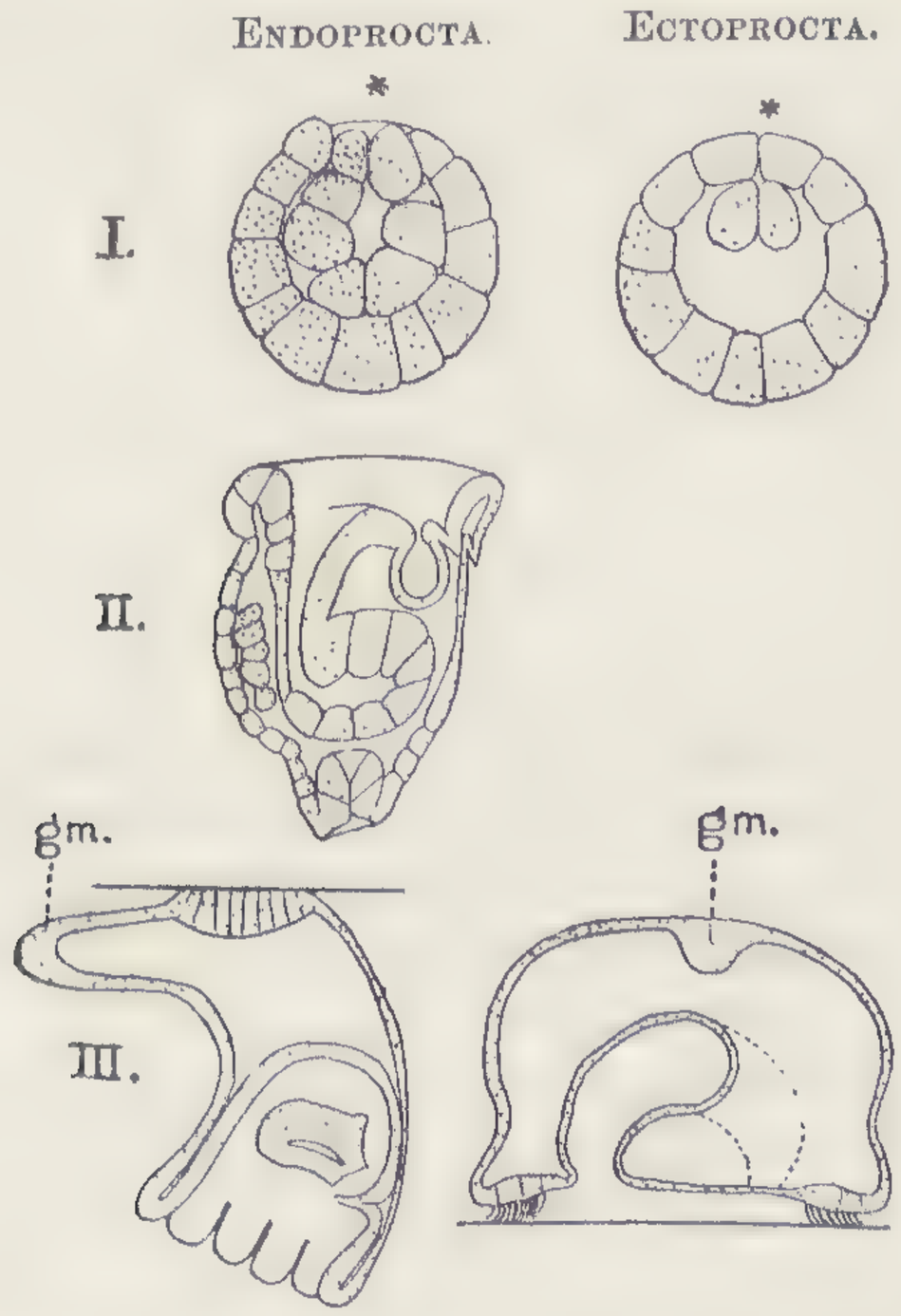
It is well known from the researches of Hatschek ('77) on Pedicellina, and of Harmer ('85) on Loxosoma, that the surface of the larva which bears the mouth and anus, i. e. its oral side, corresponds with that of the blastopore. How, then, is the oral aspect of the Ectoproct larvæ, which I have tried to show is opposite to the pole of the blastopore, to be homologized with this?

The mouth and anus of the Endoproct larva undergo a rotation after the larva has settled, so that they come to occupy the pole opposite to that at which the blastopore was. This stage of the Endoproct larva is comparable to the whole larval stage of Ectoprocta. I believe the two stages to be homologous, and that, just as polypides are *precociously* formed upon the Phylactolæmatous larva, its larval digestive tract having dropped out from the ontogeny, so the mouth and anus of Gymnolæmata are *precociously* formed on the pole opposite the blastopore, the primitive stage during which they existed at the blastoporic pole having dropped out of the ontogeny.

It is well known from the works of Harmer ('86) and Seeliger ('89),

that in Pedicellina, in which the metamorphosis of the larva has been best studied, the stolon arises from the base of the stalk — that is, at the pole where mouth and anus were first formed — at the pole of invagination. I have shown that this is true for Phylactolæmata, and probably for Gymnolæmata.

If the interpretation which I have put on Gymnolæmatous ontogeny becomes confirmed, the larvæ and the budding areas will be homologous throughout all Bryozoa. The following diagrams will explain my idea of the relation of the different ontogenetic stages in the two groups.



The left hand vertical series represents stages in the development of Endoprocta; the right hand one, stages of Ectoprocta. The blastopore (*) is throughout turned upwards in the figures. Stage I. is in both cases a young gastrula. Stage II. is that of the free-swimming larva of Endoprocta. This stage is lost in the ontogeny of Ectoprocta, in which, by abbreviation of larval life, the free-swimming stage corresponds to the condition of the fixed Endoproct after it has undergone its rotation. This stage, or one slightly later, is shown in III. Both larvæ are fixed, the Endoproct by the blastoporic, the Ectoproct by the opposite pole. The position of the stolon, or of the first polypide of the colony produced by non-sexual methods, is represented at *gm.*, near the blastoporic pole.

Summary.

The following general scheme of the budding process in Ectoprocta, derived from my own and other recent studies, may be now drawn up. The references are to pages of this paper.

All Ectoprocta build stocks or corms. The individuals in these are arranged in rows radiating from a centre, — the larva or statoblast, — and are placed one in front of another (Figs. 2, 64[a], 65[a], 67, 71[a], etc.).

New rows or branches are constantly being produced peripherally. There is no dichotomy in the branching (page 86), but the ancestral or median branch gives rise to one or more lateral branches, which in turn become median branches of their part of the stock.

The body wall and polypides of the median branch, as well as the *Anlagen* of lateral branches, arise from a pre-existing mass of embryonic tissue, the gemmiparous mass (pages 72–82). This may exist centrally of the forming region, as in Phylactolæmata, or peripherally, as in Gymnolæmata.

The anal aspect of the polypide is turned towards the gemmiparous mass (page 82).

The outer layer of the body wall in the budding region is one of rapidly assimilating and rapidly dividing tissue ; the inner layer of the body wall becomes filled with food taken from the body cavity in species in which the latter is early cut off by a partition (Paludicella, Bowerbankia, Lepralia ?) ; it shows no tendency to do so in species with a cœnocœl (Phylactolæmata, Alcyonidium).

The first impulse to the formation of the polypide is found in the outer layer of the body wall (excepting when this is highly modified, as in Cristatella), and many cells seem to be involved in its formation from the beginning (pages 8, 56).

This outer layer of the body wall is embryonic tissue, derived from the tip of the stock (margin of the corm) as in Gymnolæmata, or from the neck of pre-existing polypides, as in Phylactolæmata. It is the direct descendant of the gemmiparous tissue of the larva, which in turn has been derived from the region around the blastopore, — in Phylactolæmata certainly, in Gymnolæmata probably (pages 8, 11, 12, 69).

The inner layer of the body wall is also embryonic in the budding region, as indicated by the fact that ova arise near the neck of the polypide, in Phylactolæmata at least (page 68).

The outer mural layer becomes the inner bud layer by invagination, with or without the formation of a cavity. In the former case (many

Gymnolæmata) the mouth of the invagination pocket rapidly closes to give rise to the neck of the polypide (page 56). In the latter case, the cavity of the bud arises only secondarily by a separation of its walls (page 18).

By a rapid growth of the walls of the bud, its distal part, in which the alimentary tract is to arise, is formed. Since this rapid growth occurs earlier at the anal side than at the oral, the rectum is formed first, the stomach last (pages 19, 57).

By an approximation of the lateral walls, alimentary tract and atrio-pharyngeal cavity become separated.

The œsophagus arises as a pocket of the atrio-pharyngeal cavity, and secondarily unites with the stomach (pages 19, 58).

The lophophore arises first as two lateral thickenings of the atrio-pharyngeal wall, then as two lateral folds, whose cavity becomes the ring canal (pages 20, 58),

Tentacles appear on the ridge of the lophophoric fold thus established, and like it are formed first at the sides of the polypide, then anteriorly and posteriorly (pages 22, 59).

The posterior end of the lophophoric ridge is the last to be formed, and, in forming, it cuts off the anal part of the atrium from the inter-tentacular cavity (pages 23, 62).

The compressed intertentacular cavity becomes circular by change in position of the oral tentacles (pages 24, 62).

The ganglion arises as a depression in the floor of the intertentacular room, and becomes included in the pharynx, which is differentiated by the change in position of the oral tentacles (pages 26, 61).

Muscles and funiculi arise from the cœlomic epithelium of both the body wall and the bud (pages 27–31, 63).

The neck of the polypide may sink to a considerable distance below the general level of the body forming the "Randwulst" of Phylactolæmata or "Diaphragma" of Gymnolæmata (pages 31, 63, 103).

The atrial opening first arises at a late period by separation of the cells of the neck.

The communication plate arises in Paludicella as a circular fold of the layers of the body wall, the mesodermal cells at the centre of which become cuticularized. It is not so completely closed as to prevent communication between the cœlomata of the two individuals it separates.

The mesodermal cells of Paludicella become stored with food mate-

rial before the formation of the communication plate, and yield it up to the rapidly growing bud.

The regenerated polypides, like the marginal ones, arise in Cheilostomes in a definite position, — on the wall of the operculum from tissue left behind to give rise to the polypide, but not wholly used up in its formation. They arise wholly from the body wall, come to lie next to the "brown body," and cause its disintegration.

The more important theoretical conclusions to which I have arrived are: —

a. There is in every stock or corm of Bryozoa a mass of indifferent cell material, which is derived directly from the indifferent cells of the larva or embryo, and whose function is to form the organs of the different individuals, including the polypides. This mass by constant growth and division affords the embryonic material for lateral branches.

b. The form of the stock and interrelation of individuals is in large part controlled by food supply.

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c. The inner layer of the Phylactolæmatous larva represents mesoderm only: the entoderm has become rudimentary through loss of the alimentary function.

d. The polypides arise in Phylactolæmata at the pole of ingression, which is probably homologous with the aboral pole of Gymnolæmata.

e. The inner layer of the polypide bud is composed of cells derived from the rim of the blastopore, and they are to be regarded as still indifferent, and as first becoming differentiated into ectoderm and entoderm in the formation of the young polypide.

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f. Gemmiparous tissue is a rapidly assimilating tissue possessing large nuclei because actively assimilating, and staining deeply because full of food material.

g. The Endoproct and Ectoproct larvæ are to be compared by assuming that the act of rotation of the axes occurring in the former has been leaped over in the ontogeny, the mouth and anus arising at once on the pole opposite the blastopore.

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LITERATURE CITED.

Agassiz, A.

'63. On Alternate Generation in Annelids, and the Embryology of Autolytus cornutus. Bost. Jour. Nat. Hist., VII., p. 384.

Allman, G. J.

'56. A Monograph of the Fresh-water Polyzoa. viii + 119 pp., 11 pls. London, Ray Society.

'70. The Genetic Succession of Zoöids in the Hydroida. Trans. Roy. Soc. Edinb., XXVI. 1, p. 97.

Altmann, R.

'90. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig, Veit & Comp. 145 pp., 21 Taf.

Barrois, J.

'77. Recherches sur l'embryologie des Bryozoaires. Travaux l'Inst. Zool. de Lille, I., 305 pp., 16 pls.

'82. Embryogénie des Bryozoaires. Essai, etc. Jour. Anat. et Physiol., XVIIIe Ann., p. 124. [Transl. in Ann. and Mag. Nat. Hist., (5), X., p. 265.]

'86. Mémoire sur la métamorphose de quelques Bryozoaires. Ann. Sci. Nat., Zool., (7), I. 1, p. 1.

Braem, F.

'88. Untersuchungen über die Bryozoen des süssen Wassers. Zool. Anzeiger, XI., No. 288, p. 503; No. 289, p. 533.

'90. Untersuchungen über die Bryozoen des süssen Wassers. Bibliotheca Zoologica, Heft 6. 134 pp., 15 Taf.

Bütschli, O.

'88. Protozoa. Bronn's Klassen und Ordnungen des Thier-reichs, Bd. I., Abth. III., Infusoria, etc., pp. 1281–1584.

Chun, C.

'83. Bericht über eine nach den Canarischen Inseln im Winter 1887/88 ausgeführte Reise. Sitzungsber. Akad. d. Wiss. Berlin, XLIV., p. 1141.

Claperède, E.

'70. Beiträge zur Anatomie und Entwicklungsgeschichte der Seebryozoen. Zeitschr. f. wiss. Zool., XXI. 1, p. 137

Cori, C. J.

'90. Ueber Nierencanälchen bei Bryozoen. Lotos, XI. 18 pp.

Davenport, C. B.
'90. Cristatella: The Origin and Development of the Individual in the Colony. Bull. Mus. Comp. Zoöl. at Harvard College, XX. 4, p. 101.
'91. Preliminary Notice on Budding in Bryozoa. Proc. Amer. Acad. Arts and Sciences, XXV., p. 278.

Dumortier, B. C., et Beneden, P. J. van.
'50. Histoire naturelle des polypes composés d'eau douce, etc. Bruxelles, M. Hayez. 130 pp., 6 pls.

Ehlers, E.
'76. Hypophorella expansa. Ein Beitrag u. s. w. Abhandl. d. königl. Gesellsch. d. Wiss. zu Göttingen, XXI. 156 pp., 5 Taf.
'90. Zur Kenntniss der Pedicellineen. Abhandl. königl. Gesellsch. d. Wiss. zu Göttingen, XXXVI. 200 pp., 5 Taf.

Eisig, H.
'87. Monographie der Capitelliden des Golfes von Neapel. Fauna u. Flora Golfes v. Neapel, XVI. 906 pp., 37 Taf.

Farre, A.
'37. Observations on the Minute Structure of some of the Higher Forms of Polypi, etc. Philosoph. Trans. Roy. Soc. London, Year 1837, I., p. 387.

Freese, W.
'88. Anatomisch-histologische Untersuchung von Membranipora pilosa L. u. s. w. Archiv. f. Naturgesch., LIV. 1, p. 1.

Goette, A.
'75. Die Entwicklungsgeschichte der Unke. Leipzig, Leopold Vose. 964 pp., 22 Taf.

Grant, R. E.
'27. Observations on the Structure and Nature of Flustræ. Edinburgh New Philos. Jour., (2), III., pp. 107, 337.

Haddon, A. C.
'83. On Budding in Polyzoa. Quart. Jour. Micr. Sci., XXIII., p. 516.

Harmer, S. F.
'85. On the Structure and Development of Loxosoma. Quart. Jour. Micr. Sci., XXV., p. 261.
'86. On the Life-History of Pedicellina. Quart. Jour. Micr. Sci., XXVII., p. 239.
'87. Sur l'embryogénie des Bryozoaires ectoproctes. Arch. Zool. expér. et gen. (2), V., p. 443.
'91. On the British Species of Crisia. Quart. Jour. Micr. Sci., XXXII. 2, p. 127.

Hatschek, B.
'77. Embryonalentwicklung und Knospung der Pedicellina echinata. Zeitschr. f. wiss. Zool., XXIX. 4, p. 502.

Hincks, T.

'80. A History of the British Marine Polyzoa. 2 vols., 8°. (11) + cxli + 601 pp., 83 pls. London, John van Voorst.

Joliet, L.

'77. Contributions à l'histoire naturelle des Bryozoaires des côtes de France. Arch. Zool. expér. et gen., VI. 2, p. 193.

Joyeux-Laffuie, J.

'88. Description du Delagia chætopteri (J. J.-L.). Arch. Zool. expér. et gen., (2), VI., p. 135.

[This is the Hypophorella expansa of Ehlers, '76.]

Jullien, J.

'90. Observations sur la Cristatella mucedo, G. Cuvier. Mém. Soc. Zool. de France, III., p. 361.

Kennel, J. v.

'82. Ueber Ctenodrilus paradilis, Clap. Ein Beitrag u. s. w. Arb. zool.-zoot. Inst. Würzburg, V. 4, p. 373.

Kent, J. S.

'70. On a New Polyzoan, "Victorella pavida," from the Victoria Docks. Quart. Jour. Micr. Sci., X., p. 34.

Kleinenberg, N.

'86. Die Entstehung des Annelids aus der Larva von Lopadorhynchus. Zeitschr. f. wiss. Zool., XLIV. 1, p. 1.

Korotneff, A.

'74. Почкованіе Paludicella. Bull. Roy. Soc. Moscau, X. 2, p. 45. [Russian.]

'75. [Abstract of Korotneff, '74, by Hoyer, in Hoffman u. Schwalbe's Jahresber. Anat. u. Phys. f. 1874, III. 2, p. 369]

'87. Zur Entwicklung der Alcyonella fungosa. Zool. Anz., X., No. 248, p. 193.

'89. Sur la question du développement des Bryozoaires d'eau douce. Mém. Soc. Naturalistes de Kiew, X. 2, p. 393. [Russian.]

Korschelt, E.

'89. Beiträge zur Morphologie und Physiologie des Zellkernes. Zool. Jahrb. (Spengel), Abth. f. Anat. u. Ontog., IV. 1, p. 1.

Kraepelin, K.

'86. Ueber die Phylogenie und Ontogenie des Süsswasserbryozoen. Biol. Centralblatt, VI. 19, p. 599.

'87. Die Deutschen Süsswasser-Bryozoen. Eine Monographie. I. Anatomisch-Systematischer Teil. Abhandl. naturwiss. Verein in Hamburg, X. 168 pp., 7 Taf.

Kükenthal, W.

'85. Ueber die lymphoiden Zellen der Anneliden. Jena. Zeitschr., XVIII., p. 319.

Leidy, J.

'55. Contributions towards a Knowledge of the Marine Invertebrate Fauna of the Coasts of Rhode Island and New Jersey. Jour. Acad. Nat. Sci. Philad., (2), III., p. 135.

Maas, O.

'90. Ueber die Entwicklung des Süsswasserschwammes. Zeitschr. f. wiss. Zool., L. 4, p. 527.

Metschnikoff, E.

'82. Vergleichend-embryologische Studien. Zeitschr. f. wiss. Zool., XXXVII. 2, p. 286.

'83. Untersuchungen über die intracelluläre Verdauung bei wirbellosen Thieren. Arb. aus d. zool. Inst. Wien, V. 2, p. 141.

Nitsche, H.

'69. Beiträge zur Kenntniss der Bryozoen. I. Beobachtungen über die Entwicklungsgeschichte einiger chilostomen Bryozoen. Zeitschr. f. wiss. Zool., XX. 1, p. 1.

'71. Beiträge zur Kenntniss der Bryozoen. III. Ueber die Anatomie und Entwicklungsgeschichte von Flustra membranacea. IV. Ueber die Morphologie der Bryozoen. Zeitschr. f. wiss. Zool., XXI. 4, p. 416.

'75. Beiträge zur Kenntniss der Bryozoen. V. Ueber die Knospung der Bryozoen. Zeitschr. f. wiss. Zool., XXV. Suppl. 3, p. 343.

Nussbaum, M.

'80. Zur Differenzirung des Geschlechts im Thierreich. Arch. f. mikr. Anat., XVIII., p. 1.

'87. Ueber die Theilbarkeit der lebendigen Materie. II. Mitth. Arch. f. mikr. Anat., XXIX. 2, p. 265.

Oka, A.

'90. Observations on Fresh-water Polyzoa (Pectinatella gelatinosa, nov. sp.). Jour. Coll. Sci., Imper. Univ. Japan, IV. 1, p. 89.

Ostroumoff, A.

'86[a]. Contributions à l'étude zoologique et morphologique des Bryozoaires du golfe de Sébastopol. Arch. Slaves de Biologie, II., pp. 8, 184, 329.

'86[b]. Einiges über die Metamorphose der Süsswasserbryozoen. Zool. Anz., IX., No. 232, p. 547.

'87. Zur Entwicklungsgeschichte der cyclostomen Seebryozoen. Mitth. aus. d. zool. Stat. zu Neapel, VII. 2, p. 177.

Parker, G. H.

'89. Report upon the Organisms, etc. Rept. Mass. State Board of Health on Water Supply and Sewage, I., p. 581.

Pergens, E.

'89. Untersuchungen an Seebryozoen. Zool. Anz., XII., No. 317, p. 504.

Prouho, H.

'90. Recherches sur la larva de la Flustrella hispida (Gray), structure et métamorphose. Arch. Zool. expér. et gen., (2), VIII. 3, p. 409.

'91b. Sur le développement de la Membranipora pilosa. Assoc. Française pour l'Avanc. d. Sci., XIX., 2e part., p. 517.

Reichert, K. B.

'70. Vergleichende anatomische Untersuchungen über *Zoobotryon pellucidus* (Ehrenberg). Abhandl. königl. Akad. Wissensch. zu Berlin, a. d. Jahre 1869, II., p. 233.

Reinhard, W. W.

'80. Zur Kenntniss der Süsswasser-Bryozoen. Zool. Anz., III., No. 54, p. 208.

Repiachoff, W.

'75. Zur Entwickelungsgeschichte der Tendra zostericola. Zeitschr. f. wiss. Zool., XXV. 2, p. 129.

'75a. Zur Naturgeschichte der chilostomen Seebryozoen. Zeitschr. f. wiss. Zool., XXVI. 2, p. 139.

'78. Ueber die ersten embryonalen Entwickelungsvorgänge bei Tendra zostericola. Zeitschr. f. wiss. Zool., XXX., Suppl., p. 411.

'80. Къ Морфологіи Мшанокъ. 69 pp., 4 Tab., 8°. Odessa, 1880.

Seeliger, O.

'85. Die Knospung der Salpen. Jena. Zeitschr., XIX. 3, p. 573.

'89. Zur Entwickelungsgeschichte der Pyrosomen. Jena. Zeitschr., XXIII., p. 595.

'89a. Die ungeschlechtliche Vermehrung der endoprokten Bryozoen. Zeitschr. f. wiss. Zool., XLIX. 1, p. 168.

'90. Bemerkungen zur Knospenentwicklung der Bryozoen. Zeitschr. f. wiss. Zool., L. 4, p. 560.

Semper, C.

'77. Beiträge zur Biologie der Oligochaeten. Arb. zool.-zoot. Inst. Würzburg, IV. 1, p. 65.

Smitt, F. A.

'65. Om Hafs-Bryozoernas utveckling och fettkroppar. Öfversigt Kongl Vetenskaps-Akad. Förhandl., XXII. 1, p. 5.

'65a. Kritisk förteckning öfver Skandinaviens Hafs-Bryozoer. Öfversigt Kongl. Vetenskaps-Akad. Förhandl., XXII. 2, p. 115.

'67. Kritisk förteckning öfver Skandinaviens Hafs-Bryozoer. Öfversigt Kongl. Vetenskaps-Akad. Förhandl., XXIV. 5, p. 279.

Stuhlmann, F.

'87. Zur Kenntnis des Ovariums der Aalmutter. Abhandl. naturwiss. Verein in Hamburg, X. 48 pp.

Tullberg, T.

'82. Studien über den Bau und das Wachsthum des Hummerpanzers und der Molluskenschalen. Kongl. Svenska Vetenskaps-Akad. Handl., XIX. 3. 57 pp. 12 Taf.

Verrill, A. E.

'73. Report upon the Invertebrate Animals of Vineyard Sound, etc. Rept. U. S. Com. Fish and Fisheries, 1871–72, p. 295.

Vigelius, W. J.

'84. Die Bryozoen, gesammelt während 3. u. 4. Polarfahrt des "Willem Barents." Bijdragen tot de Dierkunde, XI. 104 pp., 8 Taf.

'86. Zur Ontogenie der marinen Bryozoen. Mitth. zool. Stat. zu Neapel, VI. 4, p. 499.

'88. Zur Ontogenie der marinen Bryozoen. Mitth. zool. Stat. zu Neapel, VIII. 2, p. 374.

Wagner, F. v.

'90. Zur Kenntniss der ungeschlechtlichen Fortpflanzung von Microstoma u. s. w. Zool. Jahrb. (Spengel), Abth. f. Anat. u. Ontog., IV., p. 329.

Zacharias, O.

'86. Ueber Fortpflanzung durch spontane Quertheilung bei Süsswasserplanarien. Zeitschr. f. wiss. Zool., XLIII. 2, p. 271.

Zoja, R.

'90. Alcune ricerche morfologiche e fisiologiche sull' Hydra. Bollettino Scientifico, XII. 3, p. 65.

'91. Quelques recherches morphologiques et physiologiques sur l'Hydre. Arch. ital. de Biol., XV. 1, p. 125. [Résumé of Zoja, '90.]

PLATE I.

ABBREVIATIONS.

cev. pyd.	Neck of polypide.	*gn.*	Ganglion.
cta.	Normal cuticula of adult body wall.	*i.*	Inner layer of bud.
		kmp'drm.	Kamptoderm.
cta.'	Cuticula secreted by tip.	*ms'drm.*	Mesoderm.
ec'drm.	Ectoderm.	*mu. pyr.*	Pyramidal muscles.
ex.	Outer layer of bud.	*œ.*	Œsophagus (pharynx).
ga.	Stomach.	*rt.*	Rectum.
gm.	Bud.	*ta.*	Tentacle.

All figures are of Paludicella Ehrenbergii.

Fig. 1. Stock of Paludicella Ehrenbergii, viewed as an opaque object. × 4.5.

Fig. 2. Diagram representing the interrelations of individuals in stock shown in Figure 1. *A–H* are individuals of the ancestral (median) branch; *a*, *b*, *c*, etc., lateral branches given off from the ancestral branch to the right; *a'*, *b'*, branches given off to the left; *α*, *β*, etc., lateral branches of second order given off in the direction of the distal end of the *ancestral* branch; *α'*, *β'*, etc., given off in the direction of proximal end; a_1', lateral branches of third order — to left.

Fig. 2ª. Diagram of another (smaller) stock. Letters have same significance as in foregoing.

Fig. 3. Cross section of branch near tip, showing the first trace of the bud of the polypide at *ex.*, *i.* × 635.

Fig. 4. Cross section of branch near tip, showing bud of polypide slightly older than in Figure 3. × 635.

Fig. 5. Cross section of slightly collapsed branch near tip, showing ingression of cells at *ex.* to form inner layer of bud. × 635.

Fig. 6. Longitudinal section of tip of branch to show cell structure. Zeiss, $\frac{1}{18}$ oil immersion, Oc. 1. × 1000.

Figs. 7, 8, 9. Optical sections (nearly in sagittal plane) of three tips of branches in successive stages of development, showing relations of young bud, *gm.*, to next older polypide. In Figure 8 the branch is slightly shrunken. × 87.

PLATE II.

ABBREVIATIONS.

cev. pyd.	Neck of polypide.	*ec'drm.*	Ectoderm.
cta.	Normal cuticula of body wall.	*gm. l.*	*Anlage* of lateral bud.
		kmp'drm.	Kamptoderm.
cta.'	Cuticula secreted by tip.	*ms'drm.*	Mesoderm.

All Figures from preparations of Paludicella Ehrenbergii.

Fig. 10. Surface view of cuticula near the end of a branch at intervals, *a* being nearest the tip, and *d* farthest from it. The branch was stained in Erlich's hæmatoxylin, the color being taken up by superficial cuticula only. × 320.

Figs. 11, 12, 13. Cross sections of the cuticula taken at different distances from the tip, to show the stainable and non-stainable cuticulæ. Figure 11 is from near the tip, Figure 13 farthest from it. × 1000.

Fig. 14. Longitudinal median (sagittal) section through the tip of a branch showing cells of tip and an early stage in the development of the polypide. × 410.

Fig. 15. Cross section of branch showing origin of lateral bud. × 635.

Fig. 16. Longitudinal section of body wall of branch through the point at which a lateral bud is originating. Polypide of ancestral branch is nearly adult. × 635.

Fig. 17. Longitudinal section of body wall from near the tip through the *Anlage* of a lateral bud. × 410.

Fig. 18. Cross section of branch showing histological conditions of *Anlage* of lateral bud. The polypide has reached a stage of development corresponding to that of Figure 36, Plate IV. × 1000.

Fig. 19. Longitudinal section through body wall from the same branch as Figure 17, but farther from the tip. Histological conditions are to be compared with those of Figure 17, which represents a less differentiated condition. × 410.

Fig. 20. Cross section of branch in which the polypide has reached a stage slightly younger than that of Figure 36. To show *Anlage* of two lateral buds with their cuboidal undifferentiated cells. × 410.

PLATE III.

ABBREVIATIONS.

An.	Anal side of polypide.	*kmp'drm.*	Kamptoderm.
atr.	Atrium.	*loph.*	Lophophore.
cev. pyd.	Neck of polypide.	*ms'drm.*	Mesoderm.
cl. mu. ret.	Young cells of retractor muscle.	*mu. par.*	Parietal muscle.
		œ.	Œsophagus.
cta.	Cuticula.	*Or.*	Oral side of polypide.
ec'd/m.	Ectoderm.	*rt.*	Rectum.
ga.	Stomach.	*vlv. cr.*	Cardiac valve.
gn.	Ganglion.		

All figures from preparations of Paludicella Ehrenbergii.

Figs. 21–25. Longitudinal sections through buds of polypides at successively older stages. The tip of the colony, and therefore the anal aspect of the polypide, is to the right in all cases. All figures × 410.

Fig. 21. Stage of Figure 37 (Plate IV.). Few nuclei in central region.

Fig. 22. Shows rapid growth of bud, chiefly at neck of polypide. The two inner cell layers are about to separate to form the common cavity of atrium and œsophagus.

Fig. 23. Beginning of formation of alimentary tract at rectum, *rt.* The row of nuclei separating the atrio-œsophageal cavity from the alimentary tract is due to the fusion of the two inner layers of the bud along this line.

Fig. 24. Rectum and stomach completed. Retractor muscles begin to form.

Fig. 25. Lophophore and young tentacles have made their appearance, and œsophagus and pharynx are separated from atrium. Beginning of formation of brain at *gn.*

Fig. 26. Part of cross section of a branch of stage of Figure 30. Parietal muscles, *mu. par.*, occupy a diameter of the section, and are attached to the cuticula. × 635.

Fig. 27. Young parietal muscle at stage of Figure 28. This is one of the pair which in a later stage are found lying together in Figure 26. × 635.

Fig. 28. Cross section of branch showing young polypide, and reticulated vacuolated cells. × 410.

Fig. 29. Bit of body wall, with cuticula separated from underlying ectoderm to show ends of parietal muscles. × 690.

BUDDING IN LEPTOZOA

PLATE IV.

ABBREVIATIONS.

An.	Anal side of polypide.	*i.*	Inner layer of bud.
an.	Anus.	*loph.*	Lophophore.
atr.	Atrium.	*ms'drm.*	Mesoderm.
can. crc.	Ring canal.	*mu.*	Muscle fibre in funiculus.
ec'drm.	Ectoderm.	*n.'*	Circumœsophageal nerve.
ex.	Outer layer of bud.	*œ.*	Œsophagus.
fun. inf.	Inferior funiculus.	*Or.*	Oral side of polypide.
fun. sup.	Superior funiculus.	*rt.*	Rectum.
ga.	Stomach.	*vac.*	Vacuole.
gn.	Ganglion.	*vlv. cr.*	Cardiac valve.

All figures from preparations of Paludicella Ehrenbergii.

Fig. 30. Cross section of polypide bud of stage of Figure 24, Plate III. The position is indicated by the line 30, Figure 24. × 410.

Figs. 31–34. Four cross sections of a branch through a young polypide, somewhat younger than that of Figure 25. Figure 31 is nearer the anal, Figure 34 nearer the oral surface. In Figure 34 that part only of the section of the polypide which lies near the body wall is represented. × 410.

Fig. 35. Cross section of branch through polypide of age of Figure 25. To show origin of tentacles and ring canal. × 410.

Fig. 36. Sagittal section of young polypide at period of closure of ganglion, *gn.* × 410.

Fig. 36ª. Bit of same polypide a few sections to one side of plane of Figure 36, showing origin of inferior funiculus. × 410.

Fig. 37. From cross section of branch showing early stage in development of the bud. × 410.

Fig. 38. From a sagittal section of nearly adult polypide, showing the two funiculi and their muscles. × 410.

Figs. 39 and 40. Two neighboring sections parallel to the body wall through a bud of the stage of Figure 23. Figure 40 lies three sections below Figure 39. Figure 39 shows the atrial cavity, formed as yet only on the anal side. Figure 40 shows the beginning of formation of the alimentary tract at the anal end. Note the vacuolated condition of the mesoderm. × 410.

Fig. 41. Polypide of about the stage of Figure 25 looked at *en face*. The anal tentacles, being turned under, do not appear. To show compressed condition of polypide, and alternating position of tentacles. Cf. Figure 77, Plate IX. × 320.

PLATE V.

ABBREVIATIONS.

an.	Anus.	*kmp'drm.*	Kamptoderm.
cev. pyd.	Neck of the polypide.	*loph.*	Lophophore.
clr. set.	Collare setosum.	*ms'drm.*	Mesoderm.
cta.	Normal cuticula of adult body wall.	*mu. par.*	Parietal muscles.
		mu. pyr.	Pyramidal muscles.
cta.'	Cuticula secreted by the tip of branch.	*of. atr.*	Atrial opening.
		rt.	Rectum.
ec'drm.	Ectoderm.	*spht.*	Sphincter.

All figures from preparations of Paludicella Ehrenbergii.

Fig. 42. Cross section of branch of age of Figure 37, Plate IV., to show origin of primary parietal muscles. × 410.

Figs. 43 and 44. Successive sections through a polypide slightly older than that of Figure 25, cut perpendicularly to the long axis of the branch. During this period the lophophore becomes more nearly circular, and its aboral ends meet oralwards of the rectum, *rt*. Figure 44 is nearer the tip of the branch. × 410.

Fig. 45. Axial section of neck and atrial opening of polypide just sufficiently developed to be capable of extrusion. Shows the collare setosum in place. × 410.

Fig. 46. Section of communication plate cut across the branch. Two sections (10 μ) above Figure 51. × 635.

Figs. 47–49. Three stages in the development of the communication plates. Longitudinal sections of the branch. In Figure 47, the polypide has reached the stage of Figure 22; in Figure 48, the stage of Figure 23; and in Figure 49, the stage of Figure 24. × 635.

Fig. 50. Longitudinal section through neck of young polypide, showing the sinking of the neck below the general surface of the body, and the method of forming the inner cuticula of neck. × 390.

Fig. 51. Cross section of branch through communication plate. The left side of the section includes the cuticula and the underlying flat ectodermal layer. The right side cuts a little lower into the mesodermal cells. × 635.

PLATE VI.

ABBREVIATIONS.

an.	Anus.	*i.*	Inner layer of bud.
can. crc.	Ring canal.	*kmp'drm.*	Kamptoderm.
cev. pyd.	Neck of the polypide.	*la. comn.*	Communication plate.
cl. rtl.	Reticulated cells.	*ms'drm.*	Mesoderm.
cta.	Normal cuticula of adult body wall.	*mu. par.*	Parietal muscles.
		mu. pyr.	Pyramidal muscles.
cta.'	Cuticula secreted by tip.	*n.'*	Circumœsophageal nerve.
ec'drm.	Ectoderm.	*œ.*	Œsophagus.
ex.	Outer layer of bud.	*rt.*	Rectum.
gm.	Bud.	*vac.*	Vacuole.
gn.	ganglion.		

All figures from preparations of Paludicella Ehrenbergii.

Fig. 52. Cross section of a branch through a polypide slightly older than that shown in Figure 36. The section passes through the brain and whole extent of the ring canal, together with its opening into the cœlom. × 635.

Fig. 53. Next section below Figure 52 of same series; showing the beginning of the circumœsophageal nerve ring. × 635.

Fig. 54. Shows connection of mesodermal cells of body wall, *ms'drm*, with those of the outer layer of bud, *ex.* × 1030.

Fig. 55. Origin of the secondary parietal muscle cells from mesoderm of body wall. × 635.

Fig. 56. Histological conditions of the budding regions. The cells have large nuclei, the mesodermal cells are vacuolated and rapidly dividing; the cells of the bud are densely granular. Zeiss, $\frac{1}{18}$ oil immersion, Oc. 1. × 1070.

Fig. 57. Normal vacuolated cell, full of food particles. × 1030.

Fig. 58. Longitudinal section of young lateral branch, showing highly reticulated character of mesoderm, and nearly complete formation of communication plate. × 410.

Fig. 59. Reticulated cell, showing one of the pseudopodia-like processes which frequently appear on them, projecting into the cœlom. × 1030.

Figs. 60–62. Three successive sections from a series across the tentacles of a polypide which has 15 tentacles, and is of about the stage of Figure 86. The odd tentacle (*) is shorter than the others, and lies opposite the rectum, *rt.* × 295.

Fig. 63. Cross section of branch through neck of polypide of about the age of Figure 36. Shows also the young pyramidal muscles. × 410.

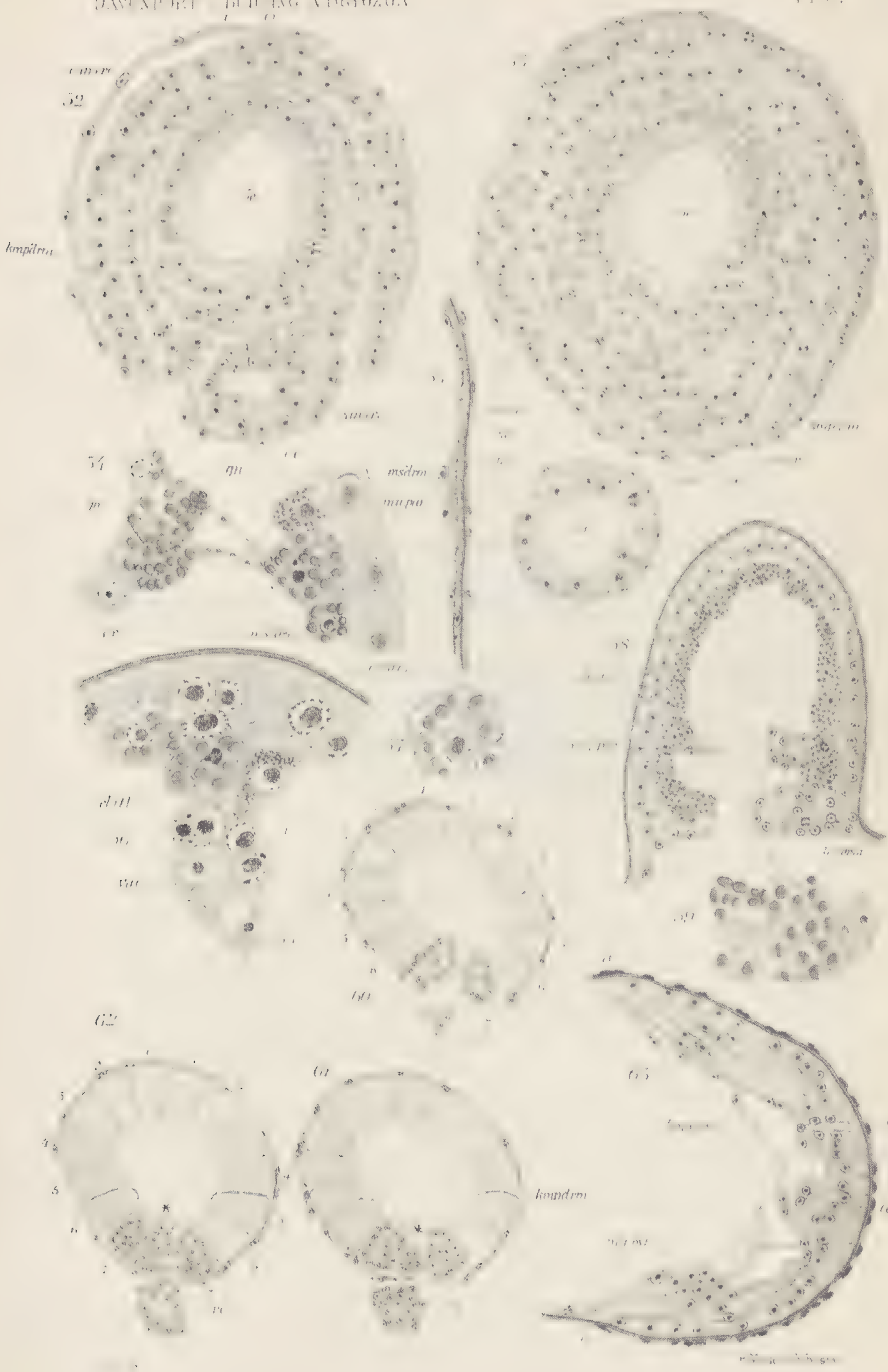
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PLATE VII.

For explanation of notation employed on this plate, see page 41.

Fig. 64. Outline drawing of one of the lateral "fans" of *Bugula turrita*, taken from the axis of the colony and spread out flat on the slide. × ca. 12.

Fig. 64ª. Diagram showing arrangement of individuals in Figure 64.

Fig. 65. Outline drawing of one of the lateral branches of a stock of *Crisia eburnea*, spread out flat on the slide. × 16.

Fig. 65ª. Diagram showing arrangement of individuals in Figure 65.

Fig. 66. Part of stock of *Bugula flabellata*. × 10.

PLATE VIII.

ABBREVIATIONS.

op. Operculum.
pyd. dyn. Degenerated polypide.
pyd. rgn. Regenerated polypide.

Fig. 67. Diagram to show interrelation of individuals in the corm, Figure 69.

Fig. 68. A part of a corm of *Membranipora pilosa*, to show regular arrangement, with a single median branch, each of whose individuals gives rise to two lateral branches. The * indicates margin of frond on which stock was growing. × ca. 8.

Fig. 69. Young corm of *Flustrella hispida*, to show arrangement of individuals. × 10.

Fig. 70. Young corm of *Membranipora pilosa*, with several median branches, showing regular arrangement. The marginal ones alone give rise to lateral branches. × 10.

Fig. 71. Young corm of *Lepralia Pallasiana*, showing arrangement of individuals. On the left, the nuclei of the cells of the body wall are shown, to indicate the inequality of their distribution. On the right, nuclei are omitted. At *pyd. rgn.* a regenerating polypide is seen, on the operculum. × 43.

Fig. 71ª. Plan of Figure 71.

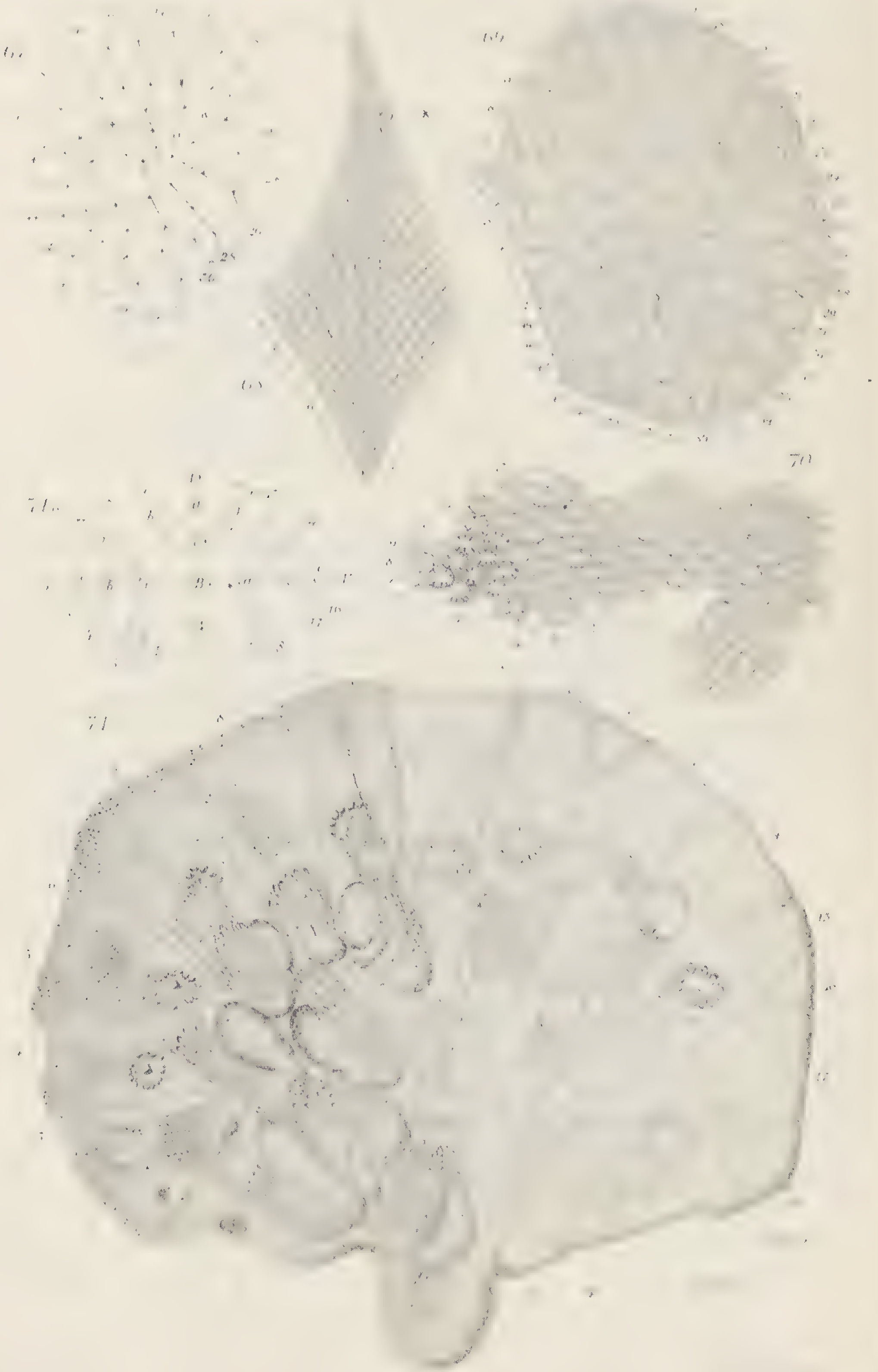

PLATE IX.

ABBREVIATIONS.

An.	Anal side of polypide.	*lu. gm.*	Lumen of bud.
atr.	Atrium.	*marg.*	Margin of corm.
can. crc.	Ring canal.	*ms'drm.*	Mesoderm.
cev. pyd.	Neck of the polypide.	*n.*	Circumœsophageal nerve.
cta.	Cuticula.	*œ.*	Œsophagus.
ec'drm.	Ectoderm.	*Or.*	Oral side of polypide.
ex.	Outer layer of bud.	*rt.*	Rectum.
ga.	Stomach.	*sep.*	Wall of zoœcium in the corm.
gm.	Bud.	*sol.*	Sole of the corm.
gn.	Ganglion.	*tct.*	Roof of the corm.
i.	Inner layer of bud.		

Fig. 72. Longitudinal vertical section through the peripheral part of the corm of *Lepralia Pallasiana*, showing the margin of the corm and two zoœcia, the older of which contains a polypide. × 160.

Fig. 73. Longitudinal vertical section through the margin of a corm of *Lepralia Pallasiana*, showing the two layers of this region and the origin of the polypide. × 410.

Fig. 74. Young regenerating polypide of *Flustrella hispida*. The section passes through the sagittal plane. × 380.

Fig. 75. Vertical section through margin of corm of *Flustrella hispida*, to show origin of polypide. × 410.

Fig. 76. Sagittal section through young polypide of *Flustrella hispida*, to show early stage of development of alimentary tract. × 410.

Fig. 77. Superficial view of young polypide from upper surface of corm of *Flustrella hispida*, showing young tentacles and their relation to the anus (at *atr.*). × 320.

Fig. 78. Bud of polypide of *Flustrella hispida* at the time of closure of the pore of invagination. × 390.

Fig. 79. Radial section through margin of corm of *Flustrella hispida*, showing bud of polypide. × 410.

Fig. 80. Young polypide of *Flustrella hispida*. × 380.

Fig. 81. Bud of *Lepralia Pallasiana* immediately before the formation of alimentary tract, showing relation of the rectal pocket (*rt.*) to the atrio-pharyngeal cavity above. × 410.

Fig. 82. Section through polypide, through lately formed brain and circumœsophageal nerves (*n.'*) growing around œsophagus (*œ.*). × 410.

PLATE X.

ABBREVIATIONS.

An.	Anal side of polypide.
an.	Anus.
atr.	Atrium.
can. crc.	Ring canal.
cev. pyd.	Neck of polypide.
cœ.	Cœcum.
cta.	Cuticula.
di'sep.	Wall of zoœcium in the corm.
ec'drm.	Ectoderm.
ex.	Outer layer of bud.
fun.	Funiculus.
ga.	Stomach.
gn.	Ganglion.
i.	Inner layer of bud.
kmp'drm.	Kamptoderm.
lu. gn.	Lumen of the ganglion.
ms'drm.	Mesoderm.
mu	Musculature of œsophagus.
mu. ret.	Retractor muscle of polypide.
œ.	Œsophagus.
op.	Operculum.
Or.	Oral side of polypide.
or.	Mouth.
pyd. dgn.	Degenerated polypide, "brown body."
rt.	Rectum.
ta.	Tentacle.

Fig. 83. Sagittal section through young polypide of *Escharella variabilis*. × 320.

Fig. 84. Regenerated polypide of *Lepralia Pallasiana* on operculum (*op.*). × 380.

Fig. 85. Cross section of pharynx of adult polypide of *Escharella variabilis*, showing perforated cell walls. × 635.

Fig. 86. Sagittal section of young polypide of *Lepralia Pallasiana*, showing formation of brain. × 320.

Fig. 87. Section parallel to sole of a corm of *Escharella variabilis* at about the stage of Figure 86, showing atrium, ganglion, and rectum. × 430.

Fig. 88. Vertical section through a bit of roof of corm of *Escharella variabilis* at neck of polypide, showing also the region of future operculum and of origin of future regenerated buds. Compare with Figure 90. × 410.

Fig. 89. Sagittal section of young regenerated polypide of *Flustrella hispida* intermediate in age between Figures 86 and 83. Shows the origin of the ganglion and rotation of the oral tentacles. × 320.

Fig. 90. Vertical section of a bit of body wall from same individual as Figure 88, to show the comparatively less embryonic condition of cells here than at neck of polypide. × 410.

Fig. 91. Operculum of *Lepralia Pallasiana* cut perpendicularly to surface, showing origin of a regenerating polypide. Body wall somewhat shrunken from cuticula. × 410.

Fig. 92. Section through a regenerated polypide of *Escharella variabilis*, showing relations of alimentary tract to "brown body" (*pyd. dgn.*). × 410.

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ectdrm
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ab
pyd dm

PLATE XI.

ABBREVIATIONS.

cev. oœ.	Neck of oœcium.	*ms'drm.*	Mesoderm.
cœl.	Cœlom.	*oœ.*	Oœcium.
ec'drm.	Ectoderm.	*ov.'*	Oöblasts.
en'drm.	Entoderm.	*pyd.*	Polypide.
ex.	Outer layer of bud.	*sto.*	Stolon.
i.	Inner layer of bud.	*tct.*	Roof of stock.
lu. gm.	Lumen of the bud.		

Fig. 93. A portion of a longitudinal section through a young stock of *Plumatella polymorpha*, about two weeks after hatching from statoblast (killed 12th May, 1890), showing the body wall just analward of the neck of a young polypide (*pyd.*), at the oral side of which a younger bud has already arisen. The inner (mesodermal) layer of the body wall shows oöblasts (*ov.'*) in various stages of development. × 600.

Fig. 94. Longitudinal section of oœcium of *Cristatella* showing embryo which is giving rise to the cœlomic epithelium by ingression of cells at its proximal pole, — i. e. the pole nearest the neck of the oœcium. There are in the next section two other cells in the cavity of the blastula, one of which appears degenerate in that it contains a huge vacuole, and has no distinct nucleus, the chromatic substance lying scattered loose near the cell wall. × 600.

Fig. 95. Longitudinal section through oœcium of *Cristatella* and its contained embryo. One polypide bud and the stolon (*sto.*) are shown here. There are two other buds in the embryo further developed than this one, lying to one side of it, and on the side of each of these buds is the *Anlage* of another. The *stolon* is seen to be well developed, lying between the ectoderm and mesoderm throughout the region bounded by the three older buds, and extending as a zone beyond them, and even beyond the *Anlage* of the youngest polypides. The embryonic tissue thus forms a disk about 75 × 150 μ in extent. × 390.

Fig. 96. Transverse section of oœcium of *Plumatella*, showing origin of first polypide. Compare with Figure 99, which represents an earlier stage. × 390.

Fig. 97. Longitudinal section through oœcium and contained embryo of *Cristatella*. The stolon is already cut off from the ectoderm. This stage immediately follows that of Figure 101, Plate XII. The forming bud is that of the first polypide. × 390.

Fig. 98. Oblique section through oœcium of *Plumatella*, showing a later stage in development of the inner layer of the larva (cf. Fig. 94). × 600.

Fig. 99. Longitudinal section of oœcium and contained larva of *Plumatella*. The bud shown at *i.*, *ex.* is the first in the colony. An incipient (second) bud is shown five sections to one side in the region indicated by an asterisk. × 410.

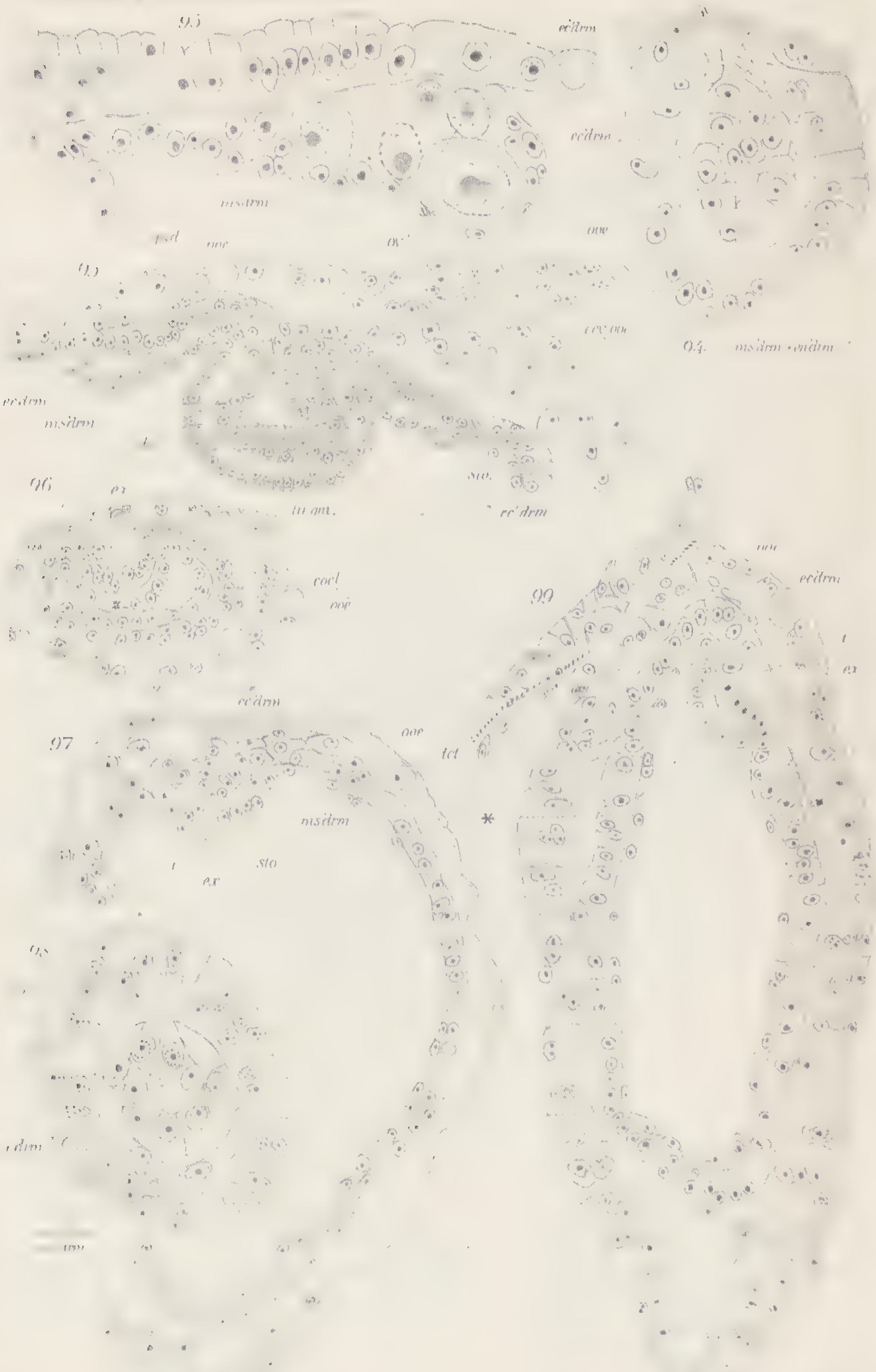
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PLATE XII.

ABBREVIATIONS.

ec'drm.	Ectoderm.	*ms'drm.*	Mesoderm.
ex.	Outer layer of bud.	*oœ.*	Oœcium.
i.	Inner layer of bud.	*sto.*	Stolon.
lu. gm.	Lumen of the bud.		

Fig. 100. Longitudinal section of a larva of *Plumatella polymorpha*, in which the two layers are established; the pole of ingression is directed upward, on the plate.

Fig. 101. Section of upper part of zoœcium of *Cristatella mucedo*, with its contained larva. Showing the formation of the stolon at the pole of ingression and the attachment of this pole to the placenta-like neck of the oœcium (*). × 390.

Fig. 102. Section through an oœcium of *Cristatella*, with its contained larva. One polypide is already established, and a second is arising. The two are the only buds in the larva. On the left of the older bud the stolon is seen to be intruding itself between the ectoderm and mesoderm of the larva. × 390.

Fig. 103. Section through the two oldest polypides of the *Cristatella* larva, together with the stolon. This larva contains one other less developed bud at one side of these two. × 390.

Fig. 104. *Plumatella polymorpha*. Stage of first bud later than that shown in Figure 96, exhibiting pore of invagination closed by overgrowth of ectoderm. × 390.

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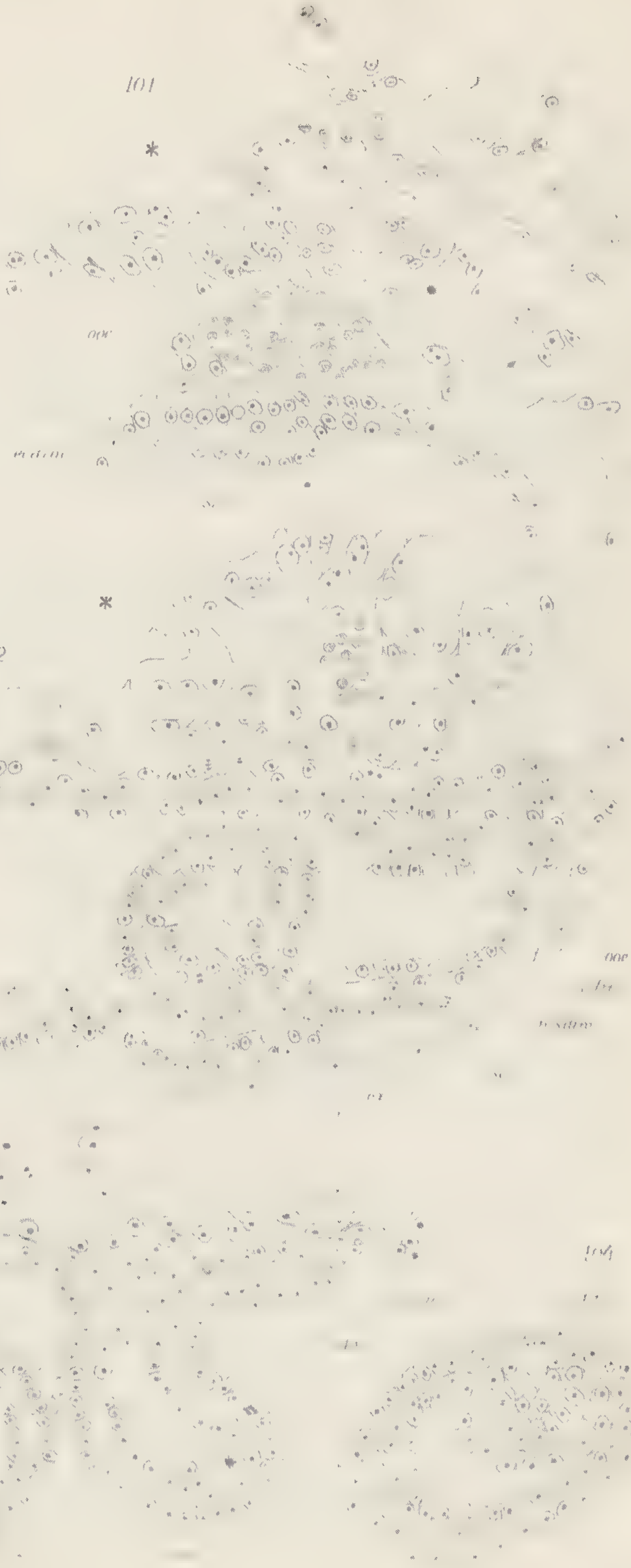

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102

No. 2. — *The Gastrulation of Aurelia flavidula, Pér. & Les.*

By Frank Smith.[1]

Preceding the appearance of Goette's ('87) publication in 1887 upon the development of Aurelia aurita and Cotylorhiza tuberculata, the gastrulation of Aurelia had been regarded, in the light of the studies of Kowalewsky, Haeckel, Claus, and others, as the result of invagination or at least of a process nearer to invagination than to any other method of gastrulation.

Goette's work seemed to show, however, that, instead of an invagination, there is an ingression of cells to form the entoderm, and that the first result of this ingression is the production of a solid gastrula, or sterrogastrula, which is only subsequently hollowed out, and is put into communication with the exterior through the formation of a prostoma at a still later period. Recently, in a paper dealing especially with the development of Cotylorhiza tuberculata, Claus ('90) reaffirms the position taken in his previous paper ('83), in which the gastrulation in Aurelia was represented as being simply a modification of invagination. In recent papers by Hamann ('90) and McMurrich ('91), Goette's views are adopted, and form part of the basis for statements that, in the development of the Scyphomedusæ, invagination, instead of being the rule, is the exception.

This want of agreement among those who have given the subject most attention makes the determination of the actual method of gastrulation in Aurelia a matter of considerable interest, and it may be assumed that any contribution to the solution of the question will not be unwelcome.

Early in the current year, at the suggestion of Dr. E. L. Mark, I undertook to investigate the method of gastrulation in A. flavidula.

Through the kindness of Mr. B. H. Van Vleck of the Boston Society of Natural History, I was enabled to spend two months of the summer of 1887 at his seaside Laboratory at Annisquam, Mass., where I then collected the material used in the present study. The embryos were killed with picro-nitric acid, and preserved in 90 per cent alcohol, in which they have been kept during the three intervening years. Of the

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy, under the direction of E. L. Mark, No. XXIX.

various staining fluids tried, Erlich's acid hæmatoxylin gave decidedly the best results for sections. For examination of the whole embryos, Grenacher's alcoholic borax-carmine and Czokor's alum-cochineal each gave good results. The latter stain possesses the peculiarity of staining embryos of different ages with corresponding degrees of intensity, the youngest stages being stained the least, the degree of intensity increasing with the age of the embryo up to the planula stage.

The result of segmentation is a one-layered blastosphere, as in A. aurita. Although the diameter of the blastocœl, or segmentation cavity, presents some individual variations at a given stage of development, it in general corresponds very nearly with that of A. aurita, as described by Goette ('87, p. 3). It increases slightly as the process of gastrulation advances. The cells of the blastosphere are usually somewhat shorter at one pole than elsewhere, and it is from this region that the entoderm is formed. The nuclei of all the cells are situated very near the outer surface of the blastosphere. Small spheroidal bodies constitute the greater portion of each cell; they are very evenly distributed through its substance, except in the vicinity of the nucleus, where they are somewhat less abundant. Vacuoles of variable sizes are usually found in some of the cells. The nuclear region stains a little more deeply than the remaining portion.

The method of gastrulation in A. flavidula is similar to that in A. aurita as described by Claus ('83, pp. 2 and 3), although it resembles even more closely a typical invagination. When the process of cleavage has resulted in the formation of a blastosphere composed of somewhat more than four hundred cells, a depression of limited extent appears in the portion of the wall which is composed of the shorter cells. From this depressed region is formed the entoderm, which develops as a single continuous layer of cells surrounding a small cavity, the cœlenteron. At the beginning of the process, and throughout its duration, the cœlenteron is in communication with the exterior by means of a narrow passage, the blastopore, or blastoporic canal. See also Explanation of Figures (Plate I. Figs. 1–4). From these figures it is apparent that only a small portion of the wall of the blastosphere is concerned in the invagination, and to that extent it must be regarded as deviating from the typical invagination, where one half of the wall of the blastosphere is infolded to form the entoderm. The cœlenteron is, however, at all stages of gastrulation, an open sac-like cavity, and therefore noticeably different from that of A. aurita, of which Claus ('83, p. 3), says: "Mit dem weiteren Nachrücken der die Mundspalte begrenzenden Zellen in das Innere des

Larvenleibes ändert sich jedoch allmählig das frühere Verhältniss zu Gunsten der Entodermfüllung, *die noch immer keine wahre Höhle, sondern eine schmale lineare, mit der Hauptachse des Leibes zusammenfallende Spalte besitzt.*"[1] With the growth of the entodermal layer, the cœlenteron enlarges, and the cleavage cavity is diminished, until finally it is entirely obliterated and the entoderm everywhere comes into contact with the ectoderm (Plate I. Figs. 4–6, Plate II. Fig. 11).

During the process of gastrulation, and also for a short time after its completion, the thickness of the entoderm, which is much less than that of the ectoderm, does not increase. Figures 5 and 6 (Plate I.) are from sections of two embryos at different stages of development. Figure 5 is from an embryo soon after the completion of gastrulation; Figure 6 is from an older stage. Since in each case the section is from the middle of its series, it follows that a decided thickening of the entoderm takes place between the stages represented by these Figures. This thickening is apparently due to an increase in the number of the cells, which are soon unable to find room for themselves except by elongation. The entodermal cells are quite different in appearance from those of the ectoderm; they are approximately spherical, and do not have as numerous spheroidal yolk bodies as the latter. Their nuclei, however, closely resemble those of the ectoderm, and usually lie in the portion of the cell nearest the cœlenteron.

As is to be seen from Plate II. Fig. 7, — a section nearly perpendicular to the blastoporic canal, — the blastopore in A. flavidula is very small. A similar condition has been shown by Claus to exist in A. aurita and by Metschnikoff ('86, Taf. X. Fig. 14) in Nausithoë marginata.

The nuclei of the cells composing the wall of the blastosphere are situated, as has been stated, near the surface of the sphere. But at about the time of the beginning of the invagination, sometimes a little earlier, a few of the nuclei are found in the deeper portion of the wall. At first there are only one or two such displaced nuclei to be observed in the whole embryo, but as development progresses they increase in number. A careful examination of sections shows that the cells to which they belong do not extend, like the remaining cells of the wall, through its whole thickness, but that they are wedged in as it were between the bases of the ordinary cells. The latter are much elongated, and from mutual pressure are prismatic, whereas the deep cells are spheroidal and project in some cases into the segmentation cavity. Since these cells are found at various intermediate positions between the outer and inner

[1] The original is not Italicized.

surfaces of the wall, I infer that they result from a process of migration inward, either at the time of cell division or independently of that process. Indeed, there is obviously no other possible source whence these cells could come, but the exact process of transfer is not easily determined. I believe that this increase in number is at first for a considerable time due exclusively to the migration of cells which once shared in forming the external boundary of the sphere, but later the division of cells which have already migrated into the deeper portion of the ectoderm undoubtedly contributes to this increase.

We have now to turn our attention to a phenomenon of considerable importance, the study of which from preserved material is, however, attended with difficulties. I refer to the ingression of cells from the wall of the blastosphere into the cleavage cavity, which begins a considerable time before the invagination commences. The latter does not take place until the number of cells forming the wall of the blastosphere has exceeded 400, whereas the ingression, as far as can be inferred from the cases which I have studied, may occur at any time after the blastosphere contains about 100 cells up to the period of invagination. The phenomenon of ingression in A. flavidula is not of constant occurrence, but when it does take place is similar to that represented by Goette ('87, Taf. I. Figs. 1–5) for the earlier stages of the blastula in A. aurita. It consists of a migration into the cleavage cavity of one or two, rarely more than three, of the cells of the blastospheric wall. With the exception that they assume a spherical form, because relieved from pressure, they are at first similar in size, as well as in nuclear and other characters, to the cells remaining in the wall.

The study of ingression upon preserved material is attended with difficulty, since in any one specimen we have the condition at only one stage of development, and cannot say with certainty what its condition has been in past stages, or what it might have been during some subsequent period. This can be determined only by studying the conditions existing in other embryos killed at other stages, and arranging all in their probable natural sequence. In view of this fact, I have sectioned and examined several hundred embryos which were killed at different stages of development. As far as possible the results obtained from these sections have been verified by the study of embryos cleared and mounted whole. Although this ingression occurs before invagination, I have deferred the discussion of it until now, because invagination is constant in its occurrence, whereas the ingression does not appear to be so; indeed, the majority of the specimens have shown no indications of it.

The subsequent history of these cells, as shown by the comparison of specimens of succeeding stages of development is both interesting and peculiar. I imagine that it is such cells as these to which Claus ('90, p. 3) refers when he says: "Ich habe den vereinzelt eingetretenen zwei bis drei Zellen, weil sie nicht regelmässig in jeder Blastula sich ablösen, der am vegetativen Pole einwuchernden Zellenmasse gegenüber keine weitere Bedeutung beigemessen, so dasse ich dieselben zwar auf einer Abbildung darstellte, im Texte aber nicht besonders erwähnte, und bin auch jetzt noch der Ansicht, dass diese auffallend kleinen Zellen wieder rückgebildet werden und überhaupt nicht zur Bildung des Entoderms beitragen." In my judgment, a part of the difference of opinion between Goette and Claus is due to the fact that there are two kinds of cells which find their way into the cleavage cavity. These are the large cells described by Goette as beginning to be formed at an early stage of the blastula, and much smaller cells, of which I shall have more to say hereafter, that make their appearance only at later stages of development. Claus seems to have seen "very small cells," and to have assumed that they were equivalent to the large cells figured by Goette. I am unable to say with certainty that the cells seen by Claus are the equivalents of those figured by Goette, but Claus assumes that they are, and I have the more reason to believe it because the large cells are of more frequent occurrence than the small ones. But if this be so, I do not understand how Claus could speak of them as "diese auffallend kleinen Zellen." But however that may be, I have reason to believe that the supposition of Claus, that they ultimately degenerate, is correct.

Soon after the ingression of a cell its nucleus undergoes changes which result in its disappearance as such, for instead of a nucleus there can be seen only one or more small, isolated, deeply stained particles, which I judge to be scattered portions of the nuclear chromatine (Plate II. Figs. 8 and 10). Even these are often wanting. I have said that this nuclear change follows soon after the ingression of the cell, because out of the numerous instances in which these cells have been present there is not one in which the nucleus retains its original condition after the cells in the wall of the blastula have given evidence, by their diminished size, that they have undergone division since the ingression took place. This conclusion is in part based on the assumption that at the time of ingression the ingressing cells are of about the same size as those which remain in the wall of the blastula. The ingressing cells sometimes persist, without any further apparent changes

than the disintegration of the nucleus, until the process of gastrulation is completed. Such cases are not as common, however, as others, where there is to be found in the cleavage cavity material which appears as though it had resulted from the disintegration of similar cells. This material has a spongy or vacuolated appearance, and contains faintly staining bodies or granules similar to those found in the ectodermic cells; it does not possess definitely circumscribed boundaries; on the contrary, it fills the cleavage cavity more or less completely, but is not of uniform density throughout. The fact that this material is not homogeneous, and that it contains granules, etc., prevents the conclusion that it has been produced as a simple secretion into the cleavage cavity, although it may have been formed in part by such a process. The frequent association of this material with ingression cells in the same specimen (Plate II. Fig. 8), and the lack of other ways of accounting for its presence, lead me to believe that it is produced by the disintegration which I have suggested.

There is another peculiarity of the development which I believe to be connected with this process of nuclear disintegration. It is this: after having once entered the cleavage cavity the immigrating cells seem to lose their power of division, and consequently do not become more numerous, while the cells composing the blastospheric wall undergo repeated divisions, as is shown by their increased number and diminished size.

The number of these immigrating cells is small, usually only one or two, very rarely more than three, so that I have not been successful in finding the "Verbindungsglieder" connecting the conditions shown by Goette ('87, Taf. I.) in his Figures 5 and 6, which Claus ('90, p. 4) regarded as essential to the substantiation of Goette's view of the method of gastrulation.

Reference has been made to the fact that in some cases the ingrowing cells persist both during and after the process of invagination. In the latter case, they are to be found in the cœlenteron rather than in the cleavage cavity. Figure 11 (Plate II.) is drawn from such a specimen. Figures 9 and 10 represent two sections of one individual in which the invagination is not completed, and furnish a hint as to the process by which the cells pass into the cœlenteron from the cleavage cavity. The entoderm being composed of less closely fitting cells than the ectoderm, doubtless admits the passage of the large immigrated cells through it more readily than the latter would (Plate II. Fig. 9). The immigrated cell is of course passive in this process. Since it is prevented by the

firm wall of the ectoderm from escaping, the pressure exerted upon it by the enlarging entoderm is probably sufficient to cause it to be forced through the entodermic wall into the cœlenteric cavity. From Figure 10 it is to be seen that one cell has already reached the gastral cavity. In speaking of these peculiarly situated cells I have thus far assumed that they are such as originally reached the cleavage cavity by an early ingression, where, with changed nuclear condition, but apparently with no further alteration, they have remained until the time of gastrulation. That this is their source is evident from the following considerations. First, the small diameter of the blastoporic canal (Plate II. Fig. 7), which is from the same series as Figures 9 and 10, precludes the assumption that they might have entered the gastrula cavity from without. Secondly, in their large size and general appearance they are unlike the cells of either ectoderm or entoderm at any time during gastrulation, and so could not have been derived from those sources during that process. Thirdly, they do correspond in size and general characters, except in their nuclear conditions, with the cells of the blastospheric wall as the latter appear at the time when ingression takes place.

It is difficult to state either the cause or the purpose of this immigration. That it is not essential to the welfare of the embryo, either by affording nourishment to the developing cells of the entoderm, or in any other way, is evident from the fact that in a large number of cases it does not occur. That it is not an inherited tendency, derived from a more primitive method of gastrulation by ingression, is probable from the fact that the immigrating cells do not appear to have any share whatever in the formation of the entoderm. On the other hand, its occurrence seems to be much too frequent to be considered as accidental.

I have stated previously (p. 119) that two very different kinds of cells are to be found at times in the cleavage cavity. Besides the large immigrating cells already described at length, I have found in a much smaller number of cases very small cells (Plate I. Fig. 2), one or two in number, that appear precisely like the deep-lying ectodermal cells already described. Because of their strong resemblance to the latter, their exceptional occurrence, and the fact that they do not appear until after the beginning of the development of the deep-lying ectodermal layer, I incline to the opinion that they are derived from that layer, and that their occurrence is entirely accidental.

At first it appeared to me surprising that two investigators could

reach such different conclusions as those published by Claus ('83 and '90) and Goette ('87), concerning the method of gastrulation in the same animal, A. aurita. Since studying this process in A. flavidula, it seems less strange. The results obtained from my first sections led me to think that the conclusions reached by Goette would be confirmed in the case of A. flavidula. Better staining, thinner sections, and more accurate orientation have made it certain, however, that the method of gastrulation in this species is much more in accord with the description given by Claus, and that the process really is one of invagination.

Certain considerations weaken my confidence in the position defended by Goette. A comparison of his Figures 6–9 ('87, Taf. I.) with some of my thicker sections, or with those which were made when the gastrula was so oriented as not to be cut parallel to the blastoporic canal, makes it appear to me probable that his results are based upon similar inadequate sections. In Figure 8 (Plate II.) there are only about one half as many nuclei visible as there are cells, the nuclei of a portion of the cells being contained in adjacent sections. In figures of corresponding stages of A. aurita as represented by Goette ('87, Taf. I.), nuclei are figured in *nearly all the cells*. I believe this to be evidence that his figures were drawn from *thick* sections. The blastopore, because of its very small diameter, is quite easily overlooked in thick sections, and especially if the plane of sectioning is somewhat oblique to the longitudinal axis of the blastopore. Since, as previously stated, the nuclei of the entodermal cells are usually situated in the portion of the cell nearest the cœlenteron, it is easy to find in thick sections of an invaginating embryo conditions like those represented by Goette in his Figures 6–8. My Figure 12 (Plate II.) reproduces a section of the same series as that represented in Figure 3 (Plate I.). The intervening section (not figured) is quite similar to Goette's Figure 8. An examination of the cells bordering the blastoporic canal in Figure 3 will show how sections like Figure 12, or such as are a little oblique to the chief axis of the embryo have the appearance of containing immigrating cells. Such sections also exhibit the flattening in the region of the shorter cells to which Goette ('87, p. 4) has called attention in the following words: "Schon während der Gastrulation zeigt sich eine Stelle des Keims im Bereich seiner kürzeren Zellen etwas abgeplattet."

Additional considerations increase the probability of the correctness of the view which I have advanced to explain Goette's error. With advancing stages of development, I have found an increase in the number of the cells composing the ectodermic wall. This is undoubtedly

subject to slight individual variations, but the number of such cells is nevertheless in quite close correlation with the stage of development. An examination of Goette's Figures 6–9 ('87, Taf. I.) reveals such a similarity in the number and size of the cells composing the ectoderm in each of the four supposed stages, that I am driven to the conclusion that they represent sections from specimens of a single stage of development, which may have been produced by cutting in planes having different relations to the chief axis of the embryo.

When we consider that in the majority of embryos there are no signs of ingression, and that in the cases where it does occur the immigrating cells in some instances degenerate early, and in others persist undivided throughout the process of gastrulation, and that they at no time show evidences of even sharing in the formation of an entoderm, — and when we further reflect that all the conditions shown in Goette's Figures 6–9 can easily be reproduced from sections of invaginating gastrulæ of a single stage of development, — it seems improbable that the entoderm of Aurelia develops even occasionally by ingression. At present, therefore, there seems to me to be no evidence that in this genus gastrulation occurs by both methods, invagination and ingression.

The Scyphomedusæ present several interesting variations in gastrulation. The anomalous development occurring in Lucernaria is as far removed from the usual process as that group itself is from the other Scyphomedusæ. According to McMurrich ('91, p. 314), the solid planula in Cyanea arctica is formed by the immigration of certain of the blastula cells. This planula is subsequently hollowed out, and gives rise to a structure like an invaginate gastrula, but it is formed without any invagination. In Cyanea capillata (Hamann, '90, pp. 16, 17) there seems to be a solid ingrowth of cells from one pole of the embryo, and a simultaneous development of the cœlenteron. The entoderm of Chrysaora (Claus, '83, p. 5, Taf. I. Fig. 21 *h*) is developed in a way which is somewhat similar to that described by Hamann for Cyanea capillata. According to Claus ('83, p. 2, and '90, p. 4), the gastrulation of Aurelia aurita approximates the method by invagination a little more closely than that of Chrysaora, since its cells are arranged in a single layer about the fissure-like cœlenteron. Aurelia flavidula exhibits a still more nearly typical invagination, since the cœlenteron is from the beginning an open sac-like cavity. Cotylorhiza tuberculata (Cassiopea Borbonica) has an invaginate gastrula which closely resembles that of Aurelia flavidula (Claus, '90, Taf. I. Figs. 2 and 3; Kowalevsky, '73, Taf. II. Fig. 1). Finally, in Pelagia noctiluca and Nausithoë marginata, as

shown by Metschnikoff ('86, pp. 66–68, Taf. X.), there is a typical invagination.

If the observations of McMurrich ('91, p. 314) on Cyanea arctica are substantiated, we have among the Scyphomedusæ one example of the formation of a sterrula by ingression, with the subsequent formation of a gastrula-like structure, without an invagination. From the preceding summary it is to be seen that there are in Scyphomedusæ two cases in which the mode of gastrulation appears to be intermediate between ingression and invagination, and at least four cases of unquestionable invagination. If, in the light of so much variation in the mode of gastrulation in this group as is shown by the few forms studied, it is safe to conclude that any one mode is typical, that mode would certainly appear to be invagination, and not, as Hamann and McMurrich have recently maintained, ingression.

Cambridge, June 20, 1891.

BIBLIOGRAPHY.

Claus, C.

'83. Untersuchungen über die Organization und Entwicklung der Medusen. Prag u. Leipzig, 96 pp.

'90. Ueber die Entwicklung des Scyphostoma von Cotylorhiza, Aurelia und Chrysaora, sowie über die systematische Stellung der Scyphomedusen. I. Arbeiten a. d. zool. Inst. Wien, Tom. IX. p. 85.

Goette, A.

'87. Abhandlungen zur Entwicklungsgeschichte der Tiere. Viertes Heft. Entwicklungsgeschichte der Aurelia aurita und Cotylorhiza tuberculata. Hamburg u. Leipzig, 79 pp.

Hamann, O.

'90. Ueber die Entstehung der Keimblätter. Ein Erklärungsversuch. Internat. Monatsschr. f. Anat. u. Physiol., Bd. VII. pp. 1--28.

Kowalevsky, A.

'73. Untersuchungen über die Entwicklung der Coelenteraten. Nachrichten Gesellsch. Freunde Naturerkennt., Anthropol. u. Ethnog. Moskau, 1873. (Russian.)

See also Hoffmann u. Schwalbe, Jahresbericht, Bd. II. p. 279.

McMurrich, J. P.

'91. Contributions on the Morphology of the Actinozoa. II. On the Development of the Hexactiniæ. Jour. Morphol., Vol. IV. p. 303.

'91[a]. The Gastræa Theory and its Successors. Biological Lectures delivered at the Marine Biol. Laboratory, Wood's Holl. Boston, p. 79.

Metschnikoff, E.

'86. Embryologischestudien an Medusen. Wien, 159 pp.

EXPLANATION OF FIGURES.

All the figures were drawn from sections with the aid of an Abbé camera. The sections from which the figures were made were 5 μ in thickness.

PLATE I.

ABBREVIATIONS.

bl'po.	Blastopore.
cav. sg.	Segmentation cavity.
cl.	Immigrated cell.
cœlent.	Cœlenteron.
cog.	Coagulum.
ec'drm.	Ectoderm.
en'drm.	Entoderm.
nl.	Chromatic portion of degenerated nucleus.
nl. ec'drm.	Nuclei of deeper portion of ectoderm.

Figures 1–4. Sections to illustrate the nature of the invagination.

Fig. 1. An early stage of invagination. × 460.

" 2. A slightly later stage than that of Figure 1. × 540.

" 3. A stage in which the invagination is well advanced. × 385.

" 4. A gastrula with invagination completed. × 410.

" 5. Section of a gastrula cut in a plane (equator) perpendicular to the axis of the blastoporic canal. × 385.

" 6. Section of an older individual through the equator, showing increase in thickness of the entoderm. × 385.

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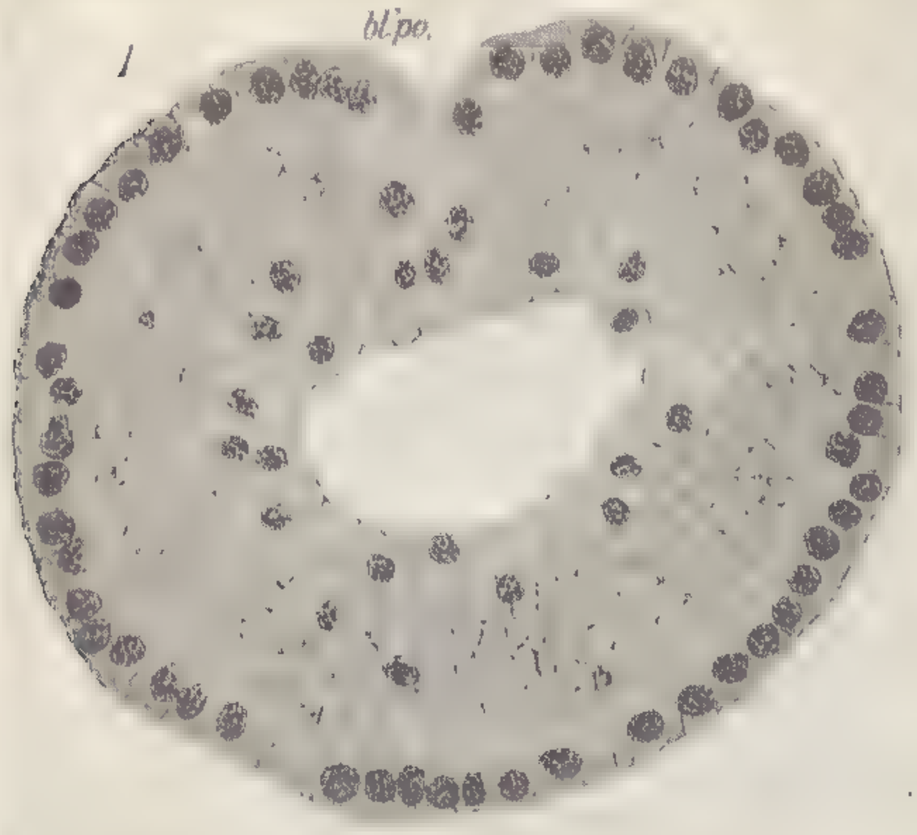

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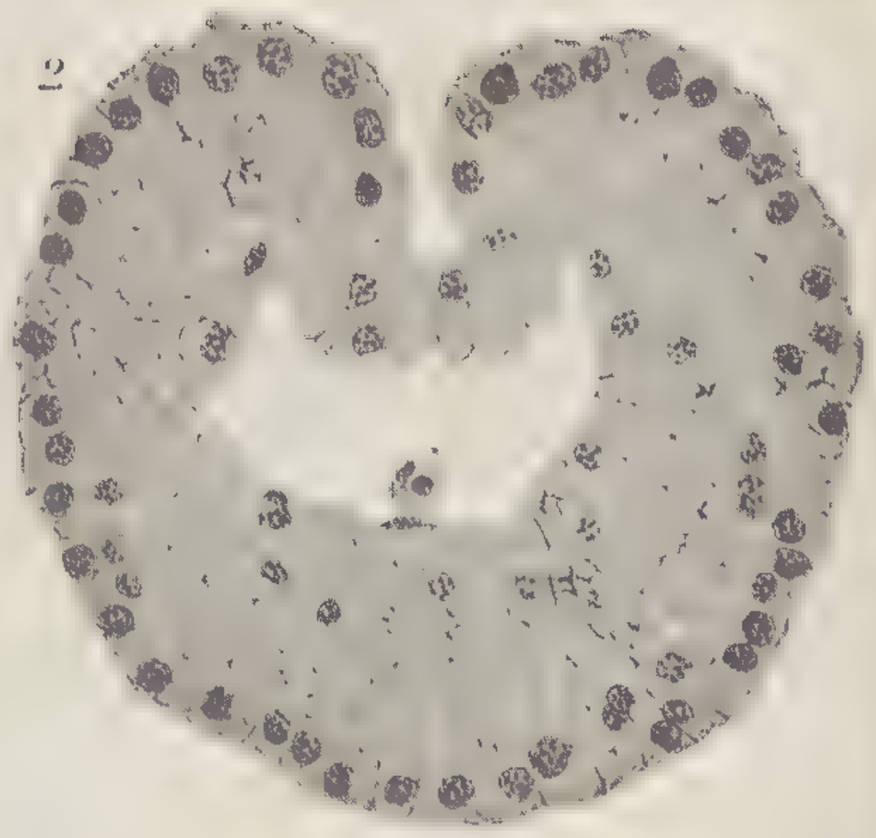

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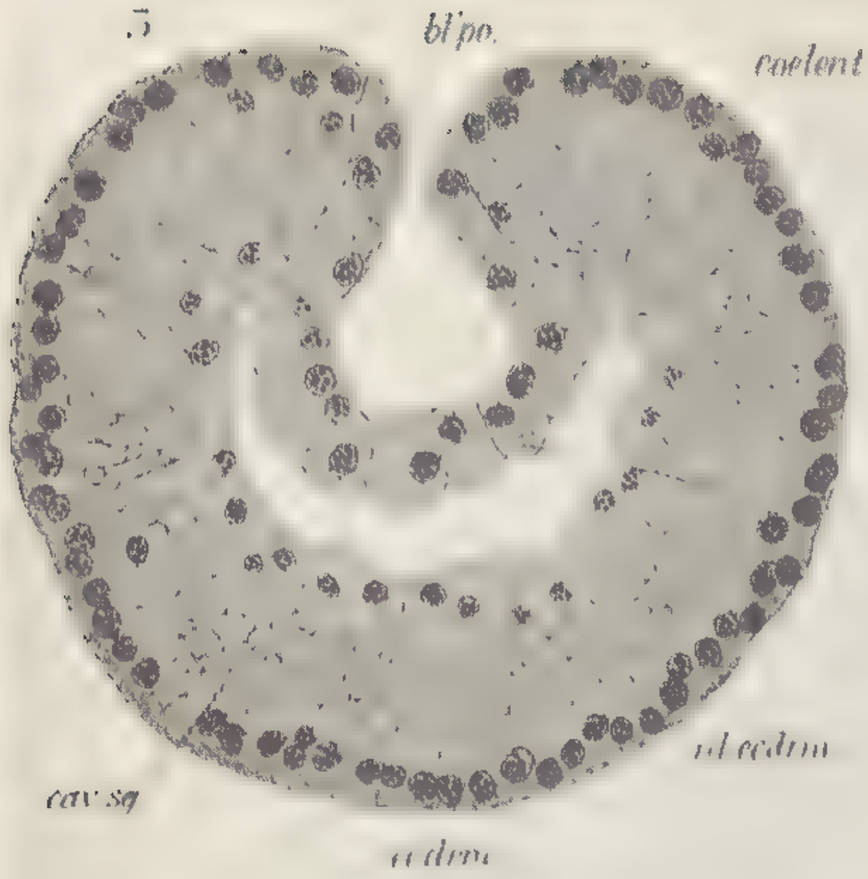

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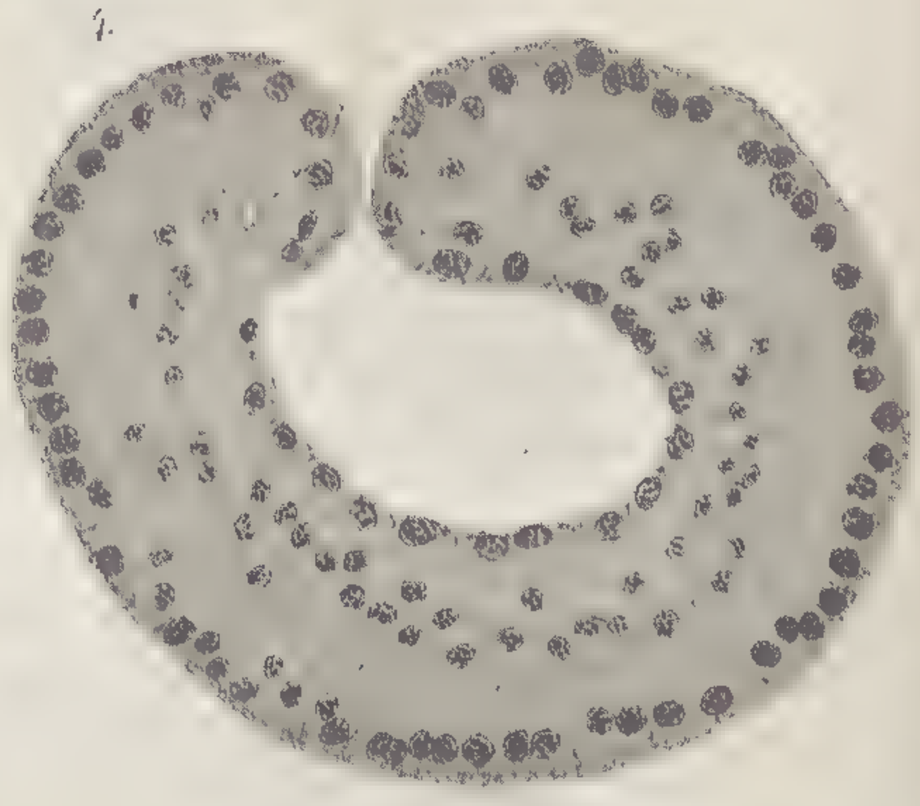

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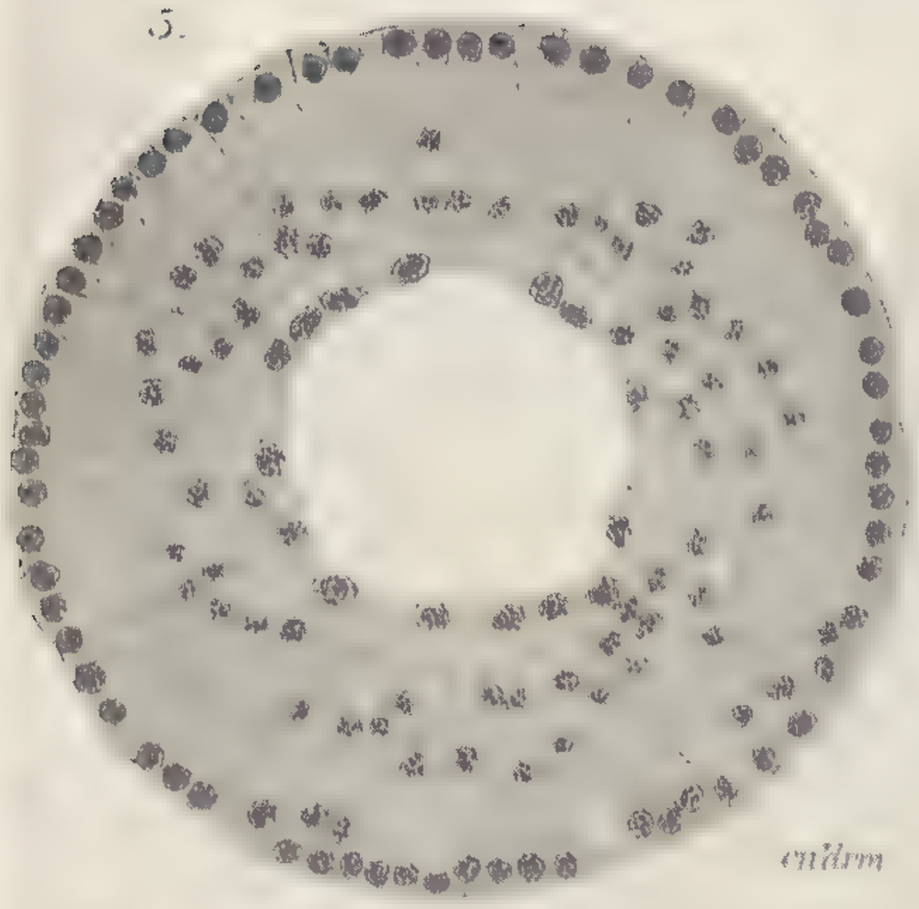

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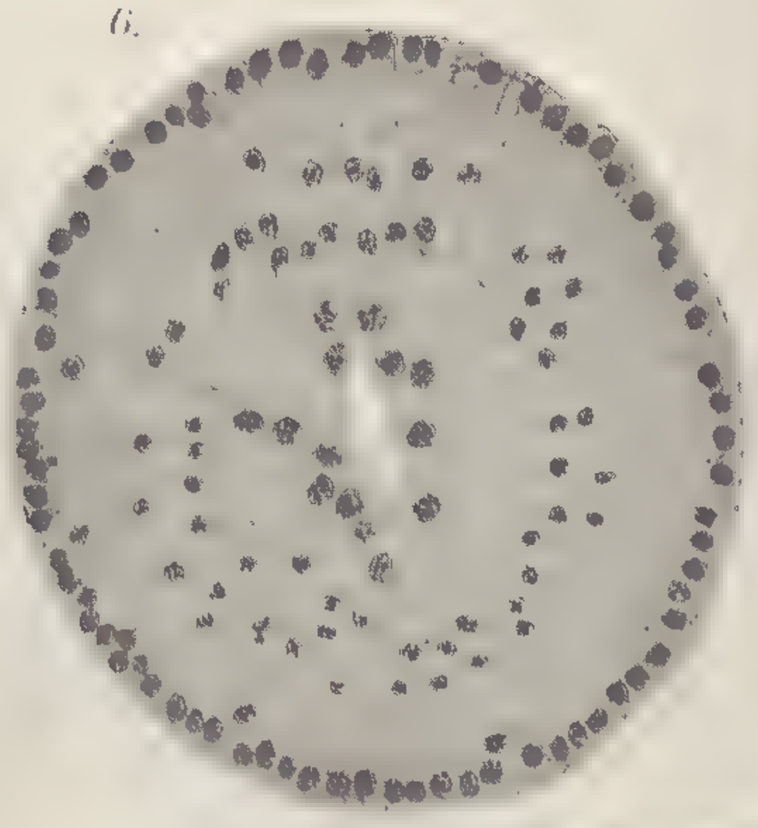


PLATE II.

ABBREVIATIONS.

bl'po.	Blastopore.
cav. sg.	Segmentation cavity.
cl.	Immigrated cell.
cœlent.	Cœlenteron.
cog.	Coagulum.
ec'drm.	Ectoderm.
en'drm.	Entoderm.
nl.	Chromatic portion of degenerated nucleus.
nl. ec'drm.	Nuclei of deeper portion of ectoderm.

Figures 7, 9, and 10 are from different sections of the same individual.

Fig. 7. Section through the blastoporic canal and nearly perpendicular to it. × 410.

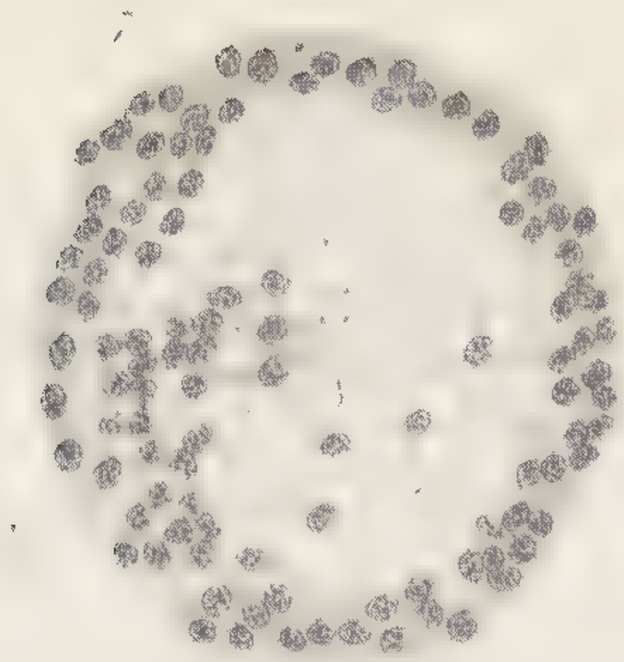
" 8. Section at a stage preceding invagination. It shows an immigrated cell in which the nucleus has degenerated. × 385.

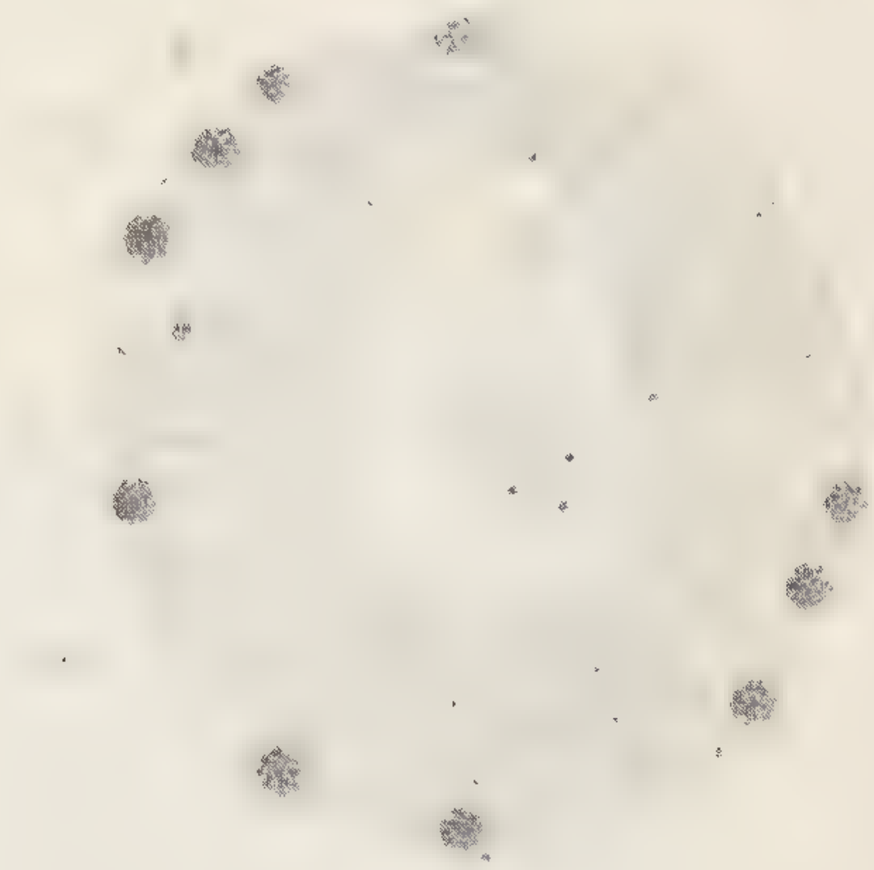
" 9. Section before the close of gastrulation, showing an immigrated cell in the segmentation cavity. × 410.

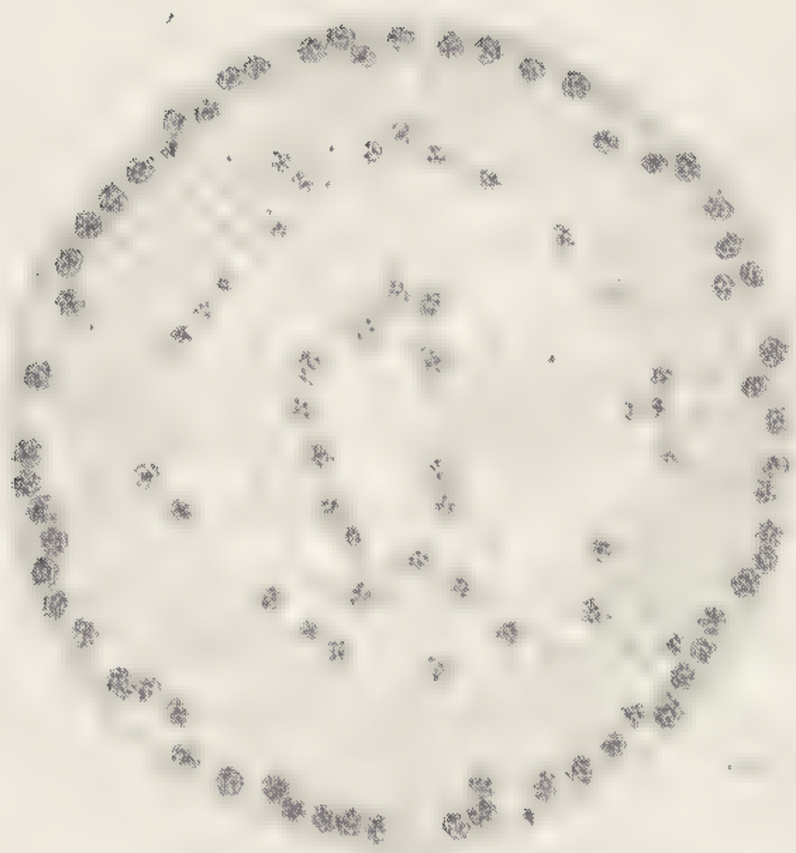
" 10. Section from the same individual as Figure 9. It contains an immigrated cell in the cœlenteric cavity. × 410.

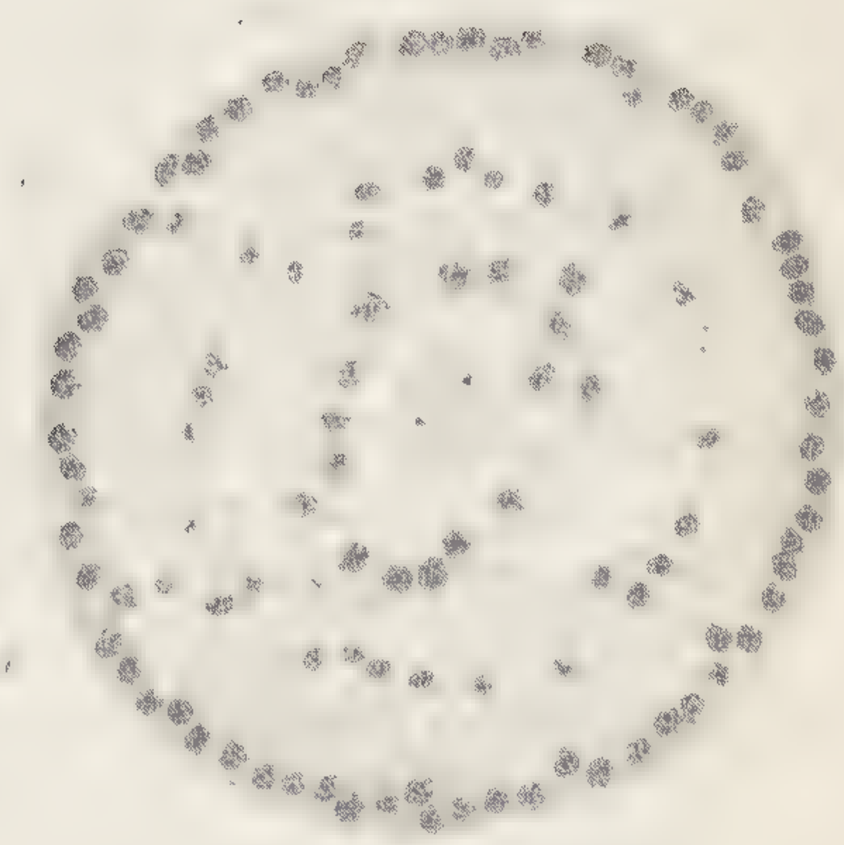
" 11. Section of a gastrula with two immigrated cells contained in the cœlenteric cavity. × 385.

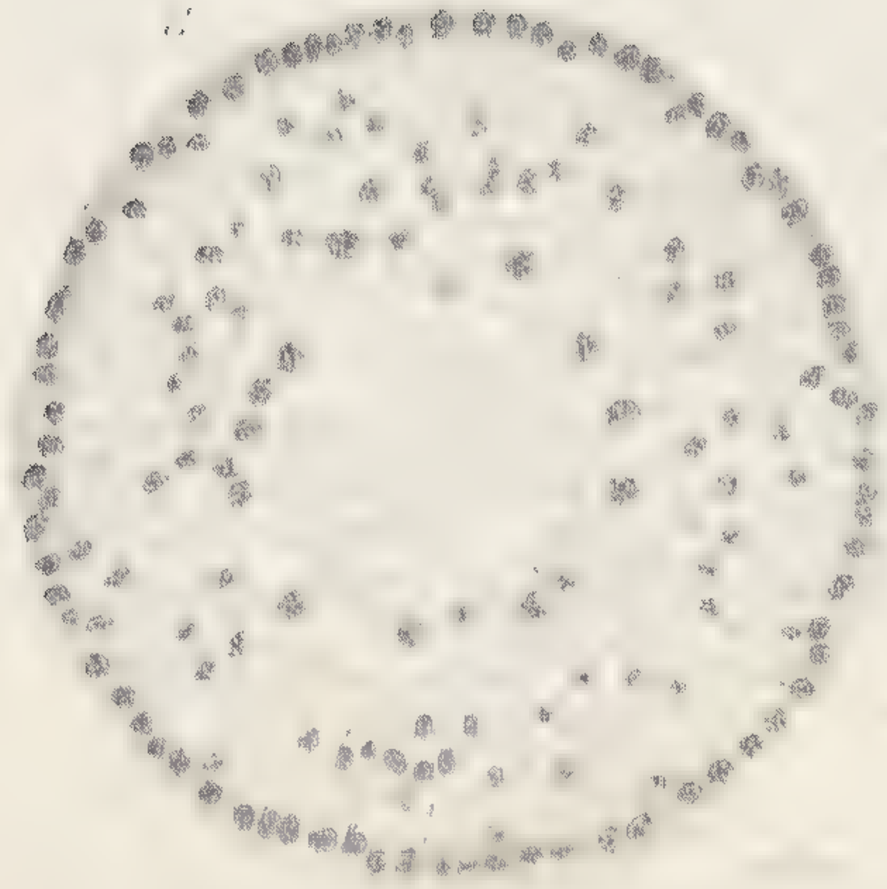
" 12. Section from the same individual as Figure 3, to show the appearance when the gastrula is cut parallel to, but at one side of, the blastoporic canal. × 385.











No. 3. — *Amitosis in the Embryonal Envelopes of the Scorpion.*

By H. P. Johnson.[1]

In the fall of 1889, at the suggestion of my instructor, Prof. E. L. Mark, I decided to work upon the problem of the so-called "direct" or amitotic division of nuclei. While in search of suitable material, my attention was called to a brief article by Blochmann ('85), describing a very well marked amitotic division for the large nuclei of the embryonal membrane of the scorpion. A number of *Centrurus* embryos were kindly given to me by my friend, Dr. G. H. Parker. These embryos had lain in 90% alcohol since the summer of 1886. The mode of fixation (for the purpose of studying the development of the eyes) was somewhat unusual; for, immediately after their removal from the mother, they were immersed in 35% alcohol, and thence carried up quite rapidly, through 50 and 70%, to 90%. Notwithstanding this rather crude method, the membranes were in excellent histological condition, in no way inferior to material afterwards prepared by the most approved methods of fixation.

In addition to the material above mentioned, I received from Mr. Richard Goeth, of Burnet County, Texas, during the following winter and spring, about three dozen live specimens of *Centrurus* (sp. incog.).[2] A lot that arrived in the latter part of May contained several pregnant females, with embryos in different stages. The scorpions were chloroformed, and the ovarian tubes with the embryos enclosed were dissected out as quickly as possible. A number of killing agents were used, including Flemming's weaker chrom-aceto-osmic, Rabl's chrom-formic, Perenyi's fluid, Kleinenberg's picro-sulphuric, and Merkel's fluid.

For staining, I have used chiefly Ehrlich's hæmatoxylin. Grenacher's alcoholic borax-carmine and Czokor's alum-cochineal have given fair results. Safranin, employed according to Flemming's method, I

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy, under the direction of E. L. Mark, No. XXX.

[2] This is the species used by G. H. Parker in his study on the development of the eyes (see Bull. Mus. Comp. Zoöl., Vol. XIII. No. 6, p. 173, 1887), and was then undescribed. I am not aware that it has since received a name.

have found less serviceable than the stains above mentioned. After staining, the preparations were dehydrated, cleared with oil of cloves, and mounted in benzole-balsam.

The embryo is enveloped by three epithelial membranes, the ovarian capsule, the *membrana serosa*, and the amnion, — named in order from without inward.

The serosa and amnion are strictly embryonic structures, analogous to the fœtal membranes of the higher Vertebrates. There are two contradictory accounts as to the manner of their formation. Possibly they do not arise in the same way in all genera of scorpions. In a brief communication by Kowalevsky und Schulgin ('86, p. 526) upon the development of *Androctonus ornatus*, it is stated that they originate as a fold from the edge of the blastoderm, the outer layer of the fold forming the serosa, the inner the amnion. The fold grows up over the blastoderm, the edges coalesce, and the membranes finally separate from the ovum. The more recent account by Laurie ('90, p. 114) states that in *Euscorpius* the serosa arises by a proliferation of the peripheral cells of the blastoderm, extends as a delicate membrane forward and backward over the egg, which it finally covers completely, and then becomes entirely separate from the blastoderm. The formation of the amnion begins when the serosa has covered about two thirds of the embryo, and, like the serosa, its origin is ectodermic. The amnion, however, "never loses its connection with the epiblast as the serous membrane has now done, but remains attached to its edges and only extends round the egg as the epiblast extends" (p. 116). Unfortunately, I have not obtained sufficiently early stages of *Centrurus* to ascertain how its membranes arise, but, in removing the latter from the embryo, I have never found the amnion attached to the ectoderm. The membrane which I have called the "ovarian capsule" I at first wrongly took to be the follicular epithelium, and under this supposition it was indicated as *e'th. fol.* in Figure 2. Like the follicular epithelium, it arises from the ovarian tube; but the follicle is formed as a diverticulum of the tube, previous to the maturation of the ovum, and serves as a nutritive capsule for the latter during its growth. The ovarian capsule, on the contrary, is that part of the ovarian tube which receives the ovum after fertilization, and enlarges to accommodate the growth of the embryo.

The fœtal membranes fit so loosely over the embryo that they can be easily removed in a single piece. In late stages, the ovarian capsule is readily separable from the membranes; in earlier stages, it adheres closely to them. It is rarely possible to separate the serosa from the amnion,

and a transverse section (see Fig. 2) shows only a trace of a dividing wall between them, although in surface view the cell walls of both membranes are clearly seen (Fig. 1). Metschnikoff (71, p. 219) describes the membranes of *Scorpio* (*Euscorpius*) *italicus* as connected with each other by delicate fibres, which terminate just over the amniotic nuclei. I have found such fibres in the earlier stages of my material, but not in the older ones, nor are they everywhere present in the younger membranes. The membranes of the Brazilian scorpion examined by Blochmann ('85, p. 481) were found closely applied to each other.

I. The Serosa.

Plate I.; Plate II. Figs. 14, 15; Plate III.

The cells of the serosa have great superficial extent, measuring half a millimeter or more in diameter; but proportionally they are very thin. Their size is exceedingly variable, as may be seen by comparing Figure 3 with Figures 11 and 13 of the same magnification, although the last two represent cells of only average size. Both small and large cells are apt to be aggregated in certain parts of the serosa, yet very small cells often occur sporadically in the midst of large ones. The cell walls are extremely distinct in late stages of the embryo, but in earlier stages are often difficult to trace in an ordinary stained preparation. As remarked by Blochmann, they have a distinct fibrous structure. The cells are irregularly polygonal in shape, usually elongated, sometimes nearly square or triangular. Not infrequently they are bounded by curved outlines (Fig. 13).

The nuclei of the serosa measure from 25 μ to 60 μ or more in diameter, but as a rule are small in proportion to the cells (Figs. 1–3 and 11–15). In the membranes of young embryos the nuclei are larger absolutely and in proportion to the cells than in old membranes. In face view the resting nucleus is nearly circular; in section, it is seen to be considerably flattened, in accordance with the thinness of the cell (Fig. 2, *nl. sr.*). It occupies the full thickness of the serosa, and sometimes causes a bulging of the cell at the point where it lies, as is shown in Figure 2. Blochmann states ('85, p. 480) that the nuclei of the serosa always cause that membrane to encroach *inward* upon the amnion; but a dividing line between amnion and serosa is so seldom visible in *Centrurus*, that I am unable to say whether such is the case.

The nuclear membrane is thin, but clearly visible, except in nuclei that have undergone degeneration. The chromatic substance, or nuclein, is

for the most part in the form of granules distributed evenly throughout the nucleus. Indications of a reticular or filamentous structure are, however, frequently present. I believe there is a chromatic network throughout the nucleus, but the abundance of granular chromatin prevents one from tracing it. Several nucleoli are always present. They are extremely variable in size and shape, and in many cases appear to be only aggregations of granular chromatin. They take a stain with hæmatoxylin and carmine in no way different from the rest of the chromatin, except that it is more intense.

A very large proportion (about four to one) of the cells of the serosa contain two nuclei. These pairs of nuclei have all arisen from single nuclei by amitotic division. It is obvious that division of the cell is not contemporaneous with, and does not immediately follow, the division of the nucleus. In many cases, especially when the embryo is far advanced, cell division probably does not occur at all. Very few cells out of the thousands I have examined have had more than two nuclei; but I have found several with three nuclei, and two cells with *four*. This seems to be the maximum number. These cells of the serosa, therefore, are not to be classed with multinucleate cells in which the nucleus divides into a great number of irregular and unequal fragments. Here the division takes place in an orderly fashion, and division of the cell follows nuclear division in regular sequence, though not immediately.

In every serosa examined, nuclei were found in process of division. Some preparations furnish many more examples of division than others; and occasionally three or four adjacent cells will contain dividing nuclei (Fig. 15). Very frequently, however, only one or two dividing nuclei will be found in the whole serosa. It cannot therefore be supposed that nuclear division is frequent; and I have found that there are more cells with dividing nuclei in the membranes of late stages of the embryo than in the earlier ones.

The first sign of approaching division is an elongation of the nucleus (Fig. 4), almost always parallel to the long axis of the cell. Naturally, the elongation progresses by insensible gradations from the nearly circular form of the resting nucleus, so that one cannot say positively that the nucleus is going to divide until the elongation has become marked. The absolute amount of elongation varies greatly, and is less in the membranes of young embryos than in those of older ones. The example represented in Figure 4 is from an old membrane, and shows almost the extreme of elongation. This stage, while giving not the slightest evidence of ordinary mitosis, is characterized by a longitudinal arrange-

ment of the chromatic substance, as indicated in Figure 4. The effect is most marked upon the nucleoli. Blochmann ('85, p. 482) found only two nucleoli at this stage, and these were usually situated one at each end of the elliptical nucleus. Where there are several nucleoli, as is usually the case with the nuclei I have studied, there is an approximately equal distribution of them to the daughter nuclei. The nucleoli vary so much in size and shape, that it is impossible to say how precise is the apportionment of chromatin by this method.

Most nuclei in the elongated condition already show a slight constriction, generally more marked on one edge than on the other (Fig. 4). If no further elongation takes place, the constriction becomes deep and narrow, as represented in Figures 5 and 12. This style of division is characteristic of young membranes, and gives rise to daughter nuclei which lie close together, or even in contact (Fig. 13). It is doubtless a more vigorous and rapid type of division than that found in the older membranes, to be described directly. If the nucleus continues to elongate while constricting, it assumes the dumb-bell form represented in Figures 6 and 7. The daughter nuclei, at first ovate or pyriform, become rounder as the connecting thread becomes thinner. Division of this type is almost confined to old membranes; I have rarely found it in those from young embryos.

The nuclei represented by Figures 6 and 7 show more clearly than usual a peculiar arrangement of the chromatic threads. The filaments have the appearance of a fascicle of slender rods, which lie very close together in the connecting bridge, and thence radiate into both daughter nuclei. They are stainable both with carmine and hæmatoxylin. Sometimes these threads can be resolved into rows of granules (Fig. 7, right-hand daughter nucleus). The later stages also show traces of these longitudinal threads (Figs. 8, 9, 10). In the example represented by Figure 6, the nucleoli partook of the general longitudinal disposition of the chromatic substance, but were probably arranged in this manner at an earlier stage of division, as explained for Figure 4. In the later stages of division, this arrangement of the nucleoli is gradually lost.

The final stages, represented in Figures 8, 9, 10, may be briefly described. These stages are far commoner than the early ones; hence, it must be supposed that they require more time. The constricted portion is drawn out into a thin, deeply staining thread. This thread undoubtedly contains chromatin, and in a peculiarly condensed form. In this respect these nuclei differ from the nuclei of the Malpighian vessels of *Aphrophora spumaria*, as described and figured by Carnoy

('85, Plate I. Fig. 7); for the connecting thread in the dividing nucleus of *Aphrophora* remains unstained, and therefore contains no chromatin.

The dividing nucleus represented by Figure 8 is peculiar in several respects. In the first place, the daughter nuclei are very unlike in form, though this is by no means unusual with dividing nuclei from old membranes. All the stainable nucleoli are in one daughter nucleus, while the other still shows a faint longitudinal arrangement of its chromatic threads. The sharply stained connecting thread is notched at a point midway between the daughter nuclei, probably indicating the place where, at a later stage, rupture would have occurred. The daughter nucleus on the left is nearly destitute of chromatin in the crescent-shaped space lying next the connecting thread, and an inner contour line is visible (x), from the central point of which a stainable cord extends to the proximal end of the connecting thread. I have seen a similar appearance in the late stages of other dividing nuclei, and it undoubtedly indicates the manner in which the daughter nuclei sometimes attain a rounded form. Occasionally, however, daughter nuclei entirely separate from each other have a conical or tapered form.

In the last stages of division, the connecting thread is drawn out to extreme tenuity (Figs. 9 and 10). So exceedingly fine does this thread become, that, with the highest power accessible to me (Zeiss's homogeneous immersion objective $\frac{1}{18}$), I could barely trace its course through the cytoplasm, though in most cases I made out that it was continuous from nucleus to nucleus. It is finally broken at or near the centre, and the proximal tips, as Blochmann suggests, are probably absorbed by the daughter nuclei. In even so late a stage as that shown by Figure 10, the longitudinal chromatic filaments are still perceptible. The right-hand daughter nucleus contains four loop-shaped bodies that strongly resemble chromosomes. They are, however, almost unstained by hæmatoxylin.

Blochmann states ('85, p. 482) that in no case did he find a division of the cell following the division of the nucleus. As already said, the great proportion of binucleate cells renders it certain that cell division is not an *immediate* consequence of nuclear division. Although I have carefully examined great numbers of binucleate cells, I have only once seen a cell wall in process of formation (Fig. 27). Yet one finds plenty of evidence that cell division does take place. Pairs of cells like those in Figure 11 are of frequent occurrence. It is safe to infer, I think, from the arrangement of the binucleate cells which surround these, as well as from the correspondence in size and shape of this pair,

that they have arisen from an elongated binucleate cell by the formation of a divisional cell wall. In one instance, I have found a cell wall *fully formed before division of the nucleus was completed* (Fig. 27). It cuts across the fine connecting thread at about the middle point of the latter. This must be considered as in some degree abnormal, especially since it was found in a serosa the nuclei of which had evidently degenerated.

Although division of the cell is almost always accomplished by the formation of a cell wall, I have found several *constricted cells*, showing that division may be partly, or even wholly, effected in this manner. Sometimes the constriction is so deep that the opposite walls meet (Fig. 28); but it is more usual to find that, after the cell has become considerably constricted, a cell wall is formed joining the inward curves of the constriction, and completing the division. At first, I thought it possible that the constriction was mechanically produced by the pressure of growing cells on either side. But this would not explain the invariable occurrence of the constriction at precisely the point where it would take place in a free cell, — equidistant from the daughter nuclei. Furthermore, the curvature of cell walls (see Fig. 13), which is almost certainly caused by the growth of cells and consequent tension, has no reference to the position of the nuclei.

As far as can be judged, the daughter nuclei are, as a rule, of equal size, and alike in shape. I have found many instances of beautifully symmetrical division (Figs. 9 and 10); but the nuclei of the serosa are not altogether exempt from the irregularities that seem to be inseparable from amitotic division wherever it occurs. Sometimes the resulting nuclei are obviously unequal (Fig. 13), even in young membranes; and in old membranes, where the nuclei have undergone degeneration, not only are the daughter nuclei extremely irregular in shape, but often very dissimilar in size.

Relations of the Nuclei to the Cell. — A very brief examination of a preparation of the serosa convinces one that the nuclei are symmetrically arranged in the cells. When there is but one nucleus, it occupies the centre of the cell; when there are two or three nuclei, each presides over a half or a third of the cytoplasm. This arrangement is so constant, that any marked deviation from it catches the eye at once. Instances of decidedly unsymmetrical arrangement of nuclei, one of which Figure 13 represents, are very unusual. As regards elongated cells, the daughter nuclei lie in the *long axis* of the cell, and at approximately equal distances from its ends. Occasionally, however, the nuclei lie in

the *short axis* (Fig. 12), and much more frequently are placed *obliquely*, as in cell *a*, Figure 14. We would suppose that, in the event of division of an elongated cell with nuclei lying transversely, the cell wall would pass longitudinally between the nuclei; but I have not been able to find evidence of longitudinal divisions. From the large number of cells with nuclei lying obliquely, one would infer that oblique division of the cell often took place. I am unable to discover, however, that such is the case; and it seems extremely probable that the divisional plane of the cell does not always coincide with that of the nucleus.

I have found about 25 cells of the serosa with three nuclei. This seems to be a matter of individual variation in the make-up of the membrane, for all but three of the trinucleate cells were in membranes from the brood of a single scorpion, and membranes from some broods appear to have none. I have in one instance found a *group* of trinucleate cells (Fig. 14, *1*, *2*, *3*, *4*). At this spot nuclear multiplication has outstripped cell multiplication. It is nearly always easy to see which of the two original nuclei has divided, for we find two of the nuclei smaller than the third, and nearer to each other than to the latter. In cell *2*, for instance, the pair of nuclei on the left have arisen from a nucleus occupying a position about midway between them. The same statement would doubtless hold true for the two nuclei on the right in cell *3*, and here the odd nucleus is elongated. When the cell is long and the nuclei all lie in the longitudinal axis, as is the case in cell *1*, it is usually impossible to determine which of the two original nuclei has divided; for the nuclei are equidistant, and nearly alike in size. Another type of equidistant nuclei is shown in cell *4*, — a distribution quite as characteristic of very large, broad cells as the linear arrangement is of elongated cells. I have spoken of the division of one of the two original nuclei as though it always took place *after* the nuclei were completely separate, and had taken their positions in the cell. This seems to be the usual method, for I have several times found one of the original nuclei in the act of dividing. But it is possible, of course, for them to arise by a tripartite division, in which the three nuclei would be formed simultaneously. I have found only one instance of a true triple division, represented in Figures 29 and 30, and as this occurred in a serosa which had plainly undergone degeneration, I do not consider it as altogether normal. It will be noticed that the original nucleus became trilobed, and that the lobes became daughter nuclei of approximately equal size by the formation of three divisional planes, meeting at the centre of the original nucleus. The daughter nuclei on

the right are still united to each other by strands at the corners. Very similar tripartite divisions were found by Overlach ('85, Plate XI. Figs. 35 and 41) in the epithelium of the cervix uteri. In two other cases, I have found one of the daughter nuclei in a late stage of division (Figs. 31, 32) *itself* elongating and undergoing constriction. It will be noticed that the constricted daughter nucleus is considerably larger than its mate.

I have found but two cells with more than three nuclei, and these both contained four. This condition is brought about by the division of both nuclei of a binucleate cell. On *a priori* grounds, one would reason that quadrinucleate cells would be nearly as abundant as those with three nuclei, for, apparently, it must often happen that a pair of daughter nuclei, arising as they do by a symmetrical and accurate constriction, are ready to divide at almost the same moment. Yet there are doubtless influences which operate to prevent the division of one of the nuclei. Although it is of course impossible to generalize on the characteristics of quadrinucleate cells, it may be of interest to mention the peculiarities of the two found. They are both large cells, of nearly equal width at the ends, and the breadth of both exceeds half the length. In one, both pairs of nuclei lie transversely, showing that the second divisional plane was at right angles to the first. In the other, represented in Figure 33, the lower pair of nuclei lie in the longitudinal axis, the upper pair almost transversely. One of the quadrinucleate cells is considerably larger than any cell near it, while the other (Fig. 33) though by no means small, is of much less dimensions than the immense bi- and uninucleate cells around it. I am unable to assign any reason for the multinuclear condition of this cell. One fact, however, is worthy of note. The united volume of its four nuclei does not exceed the bulk of the *single* nucleus of a neighboring cell. One cannot, of course, ascertain what the size of the primitive nucleus of the multinucleate cell was, but it is very improbable that it exceeded in volume the nucleus of the uninucleate cell in question, for the latter cell is considerably the larger of the two, and throughout this serosa the size of the nuclei bears a direct ratio to the size of the cells.

As regards the influence or influences impelling nuclei to divide independently of the division of the cell, nothing very definite can be stated. It is certain that the *absolute* or *relative size* of *the cell has little or no influence upon the division of the nucleus.* There are cells of all sizes, from the largest to the very smallest (Fig. 3), which are binucleate; and it is usual to find, side by side with bi- or multinucleate cells,

others with a single nucleus that are actually larger than the former (compare the cells in Figure 14). In such cases, the single nucleus is always larger than the daughter nucleus of the other cells. I am unable to see that multiplication of nuclei in the cell leads to any immediate increase of nuclear material. The more they divide, the smaller they become. Probably the most important office of division is *a more extensive distribution of nuclei throughout the cytoplasm, with corresponding increase of nuclear surface;* and this, considering the great superficial extent of the cells, and the comparatively small size of the nuclei (at least in the older membranes) must be a matter of some importance for the activities of the cell. It is especially so in the case of *elongated* cells. If such cells have but a single nucleus, a large part of the cytoplasm must be remote from it; and if the nucleus is at the centre of the cell, the cytoplasm at the ends of the cell will be most remote. So, to restore the equilibrium between cytoplasm and nuclei, the nucleus must elongate in the longitudinal axis of the cell, and the daughter nuclei move toward the ends of the cell.

As a matter of fact, nearly all elongated cells have two nuclei, and these lie in the long axis of the cell, usually rather nearer its ends than to each other. It cannot be denied that many short or squarish cells also contain two nuclei; and, conversely, a few much elongated cells can be found that have but one. In the latter case, it is interesting to observe that almost invariably the nucleus has begun to elongate in the longitudinal axis of the cell, and is often far advanced towards division. We can say almost with certainty, then, that such cells are of recent formation, and that the equilibrium between cytoplasm and nucleus is promptly restored by division of the latter. It is true that cases like that represented in Figure 12, where nuclear division takes place in the short axis of an elongated cell, cannot be explained in this manner. Such instances are so rare that they might almost be considered as abnormal; but the difficulty of the matter lies in the fact that we get *all gradations* between nuclei ranged in the true longitudinal axis, and those placed in the transverse axis. It is common to find them lying more or less obliquely in the cell, though the obliquity is seldom so great as to prevent them from practically fulfilling the conditions of the hypothesis.

It is not supposable that all the agencies impelling nuclei to divide, and controlling the direction in which division shall take place, reside in the cytoplasm; possibly the most potent of them exist in the nucleus itself. That axial differentiation, with definite pole and antipole, is as

characteristic of the resting nucleus as of the mitotic nucleus, was postulated by Rabl ('85, p. 323) from a careful study of the chromatic network in the "skein stage" of mitosis. In a recent paper ('89, pp. 23, 24), the same writer states that the "polar depression," usually visible in young daughter nuclei, persists much longer than usual in the epithelial nuclei of the Triton; so that for these mitotically dividing nuclei it is highly probable that polar differentiation is *always* present in the resting state. Carnoy ('85) has shown that, in the resting nuclei of the testicular cells of certain Arachnids, the chromatic filaments are distinctly arranged with reference to a definite axis (Planche V. Figs. 165–169), and Van Gehuchten ('89) has found the same in glandular cells of a Dipterous insect, *Ptycoptera contaminata.*

It is obvious that the discovery of an "organic axis," as Van Gehuchten calls it, in amitotically dividing nuclei is more difficult, for here there is no polar depression or longitudinal arrangement of chromatic filaments to indicate its direction in the resting nucleus. It is usual for each division of the nuclei of the serosa to take place at right angles, or nearly so, to the plane of the previous division. This is well seen in many multinuclear cells, where one or both pairs of nuclei lie transversely in the cell, and therefore at right angles, or nearly so, to the direction of the first division (see cells *2* and *3*, Fig. 14). In other cases, however, two consecutive divisions take place in the same direction (Fig. 14, cell *1*). It occurred to me that possibly there was an organic axis in the nuclei of the serosa which in some cases exerted a controlling influence upon the direction in which division took place, but which in most instances was counteracted by influences resident in the cytoplasm. Transverse divisions of the nucleus (Fig. 12) could then be accounted for by assuming that the influence of the organic axis is dominant in these cases, while oblique divisions would be explainable on the ground that neither influence was predominant, but that *both* acted with about equal force in directions at right angles to each other. A question of interest in this connection is, whether, when the cytoplasmic influence is dominant, and tends to make the nucleus divide in a plane parallel to its organic axis, division actually does take place in that direction. If such were the case, an organic axis would be a fact of slight morphological importance, and the longitudinal arrangement of chromatin, which takes place in the earlier stages of constriction (Figs. 4, 6, 7), might occur in any direction, without reference to an organic axis. If, on the contrary, it were necessary that the longitudinal filaments should be arranged *parallel* to the organic axis, in order that

division might take place *transversely* to the axis, this result could still be attained by a *rotation of the nucleus*, even when the tendency was for the nucleus to divide at right angles to the previous division. It is obvious that rotation would occasionally be apparent, provided it took place soon after division, and *previous* to the absorption of the proximal end of the connecting filament. I examined a large number of preparations to find evidence of rotation, but I must admit that the evidence was slight, and hardly sufficient to establish the hypothesis which I had formulated. It is therefore put forth provisionally, in the hope that it may lead to further investigations in this line.

The most striking instance of rotation was found in one of the quadrinucleate cells (Fig. 33, nuclei *a* and *b*). It is evident that three nuclear divisions have taken place without any division of the cell, producing two, three, and four nuclei. The arrangement of nuclei makes it reasonably certain that the *lower* pair arose by division of one, and the *upper* pair by division of the other nucleus of the binuclear stage. Only under this supposition could the daughter nuclei of that stage have had the normal arrangement, to which all the neighboring cells rigidly conform. We further find, that, while the upper pair of nuclei has arisen by a division in the long axis of the cell, the lower pair has been produced by division in the transverse axis, and therefore in conformity with the law previously stated (p. 136). One nucleus of each pair (*a* and *b*) retains a remnant of the connecting filament, which is directed, not toward the sister nucleus, but to a point 90° distant from it. This condition could have been brought about only by rotation of the nuclei, which in both cases has been through an arc of 90°.

In the serosæ from older embryos, the daughter nuclei almost invariably recede from each other in the course of division. The amount of recession is governed by the length of the cell (Fig. 15). In the younger membranes, as already stated, the constriction is deep and narrow, so that the nuclei not infrequently lie very near together (Fig. 13). In these young membranes, however, the nuclei are larger, and the cells are usually smaller, than in the old membranes. Since, moreover, the *large* binucleate cells of young membranes almost always have their nuclei symmetrically placed at the ends, it is probable that the nuclei gradually move apart *after* division, as the cell increases in size.

It will be seen that my interpretation of the primary cause of the division of these nuclei agrees in part with the hypothesis advanced by Chun ('90) for the explanation of amitotic division in general. This is,

in brief, that the object of amitotic division is the distribution of nuclear material throughout the cytoplasm, with corresponding increase of nuclear surface. He considers it the final phase of a series of conditions which begins with a simple lobed nucleus, and includes branched nuclei of various degrees of complication. In support of this interpretation, Chun lays stress on the statement that cell division, after an amitotic division of the nucleus, has seldom or never been observed with certainty, thereby implying that amitosis cannot have in view the multiplication of cells. I do not consider this as essential to the hypothesis, nor, in fact, do I believe him correct on this point. The evidence of cell division after amitosis seems to me abundant and conclusive. It was observed by F. E. Schulze ('75) in *Amœba polypodia;* by Ranvier ('75), Bütschli ('76), Flemming ('82), Arnold ('87), and others, in leucocytes; by Kükenthal ('85), in the lymphoid cells of Annelids; and by Carnoy ('85), in various cells of Arthropods. As the foregoing shows, there is abundant evidence that, in the serosa of the scorpion, division of the cell *sometimes*, at least, follows amitotic division of the nucleus. Furthermore, the extremely regular and well ordered manner in which the nuclei divide, and the similarity as to size and shape of the daughter nuclei, seem to me decidedly against the notion that the *sole* object of the division is to disseminate nuclear substance in the cytoplasm; for in those cases where amitosis is not followed by division of the cell, and assumably takes place simply for the purpose of dissemination, the nuclear products are very variable as to number, size, and shape.

II. The Amnion.

Plate I. Figs. 1 and 2; Plate II. Figs. 16-20.

The amnion is much thinner than the serosa, and like it is composed of a single layer of flat, polygonal cells (Fig. 1, *am.*). But, while both the cells and nuclei of the serosa have become enormously larger than the blastodermic cells from which they originated, those of the amnion have changed little as regards size. The boundaries of the amniotic cells are not always visible, and I find that preparations, even when hardened and stained in the same manner, show the greatest variation in this respect. As a rule, the cell walls in the amnion are sharply and clearly defined *only* in preparations of membranes from advanced embryos. The same is true of the cell walls of the serosa.

In general, the amniotic cell has but one nucleus, which usually occupies the centre of the cell. Blochmann makes the same statement as to

the number of nuclei in each cell, and he found no evidence of division among them. The outline of the nuclei, which measure about 15 μ in diameter, is frequently somewhat irregular or lobed. Like the nuclei of the serosa, they are flattened tangentially (Fig. 2, *nl. am.*); but notwithstanding this, they cause an outward bulging of the cell upon the serosa, as shown in Figure 2. They contain always one or more highly refractive, deeply staining nucleoli. The rest of the scanty chromatic substance is in the form of minute granules, occasionally arranged partly in a very faint network (Fig. 18, *b* and *c*). As in the nuclei of the serosa, chromatic threads frequently unite the nucleoli.

Division of the amniotic nuclei is of rare occurrence. In only one of my preparations are dividing nuclei at all abundant. The division takes place without mitosis, but is of a different type from that of the nuclei of the serosa. The only alteration of the chromatin is possibly a change in the position of the nucleoli; I have not been able to detect any modification of the reticulum. The first sign of approaching division is elongation of the nucleus (Figs. 16 and 18, *a*). A deep narrow constriction appears at the equator of the nucleus (Fig. 17). This is followed by the formation of an equatorial septum, at once partitioning off the nucleus into two daughter nuclei (Fig. 18, *b*). If there are but two nucleoli, it is the rule to find one in each daughter nucleus; but where there are several, they are often unequally apportioned. After the formation of the septum, the daughter nuclei still adhere to each other, and division seems always to be attained by deepening of the equatorial constriction in the plane of the septum (Figs. 18, *b*, *c*, and 19). I have not found any evidence of a recession of the nuclei before division of the cell. Furthermore, the rarity of binucleate cells makes it very probable that cell division follows nuclear division promptly. As in the serosa, division of the cell takes place by the formation of a cell wall without marked constriction (Fig 20). The position of the nuclei in this figure, and the frequency with which nuclei are found near the boundaries of the cells (Fig. 1, *am.*) is evidence of the promptness of cell division after the division of the nucleus.

It is clear that Chun's hypothesis will not hold in this case, for there is even less tendency than in the serosa to accumulate nuclei in the cell. This may be owing in part to the *shape* of the cell, for it is seldom elongated. It would seem that, in case the cell becomes elongated, nuclear division takes place and the cell divides immediately after the nucleus. The orientation of the nuclei with reference to the cytoplasm of their respective cells would then be accomplished by their migration to the centre of the cells.

III. The Ovarian Capsule.

Plate II. Figs. 21-26.

The epithelium of the ovarian capsule is not often easily made out in ordinary stained preparations, for the nuclei of muscle fibres and connective-tissue cells lie not only just external to the epithelial nuclei, but frequently in the same plane with them. In most of my preparations the boundaries of the epithelial cells cannot be seen at all, and I have therefore confined my attention mainly to those which show them distinctly. In shape, the cells are more or less irregular, oblong hexagons (Figures 24 and 25 represent typical shapes). The cell walls are broad and fibrillated, like those of the serosa, though the cells themselves are smaller even than those of the amnion. The nuclei are not only larger in proportion to the cells, but often larger absolutely, than the amniotic nuclei. The amount and arrangement of the chromatin in the capsular nuclei (except in a certain phase) is almost precisely like that already described for the nuclei of the amnion, but there is usually only one conspicuous nucleolus. The small amount of chromatic substance, aside from the nucleolus, has a granular appearance, but sometimes shows indications of a filamentous or reticular arrangement (see Figs. 21, 23, 24). Seen in face view, the nuclei are circular, and have a distinct nuclear membrane. The section (Fig. 2, *nl. fol.*) shows that they are less flattened than the amniotic nuclei.

Here, again, we have amitotic division, and of precisely the same type as prevails in the amnion. Apparently, division is not of common occurrence, for I have been able to find only a few instances, and have, unfortunately, not seen its earliest stages. Figures 21, 22, and 23 show the simple manner in which it is effected. As each daughter nucleus contains a nucleolus, and the ordinary resting nucleus has but one, division of the nucleolus must precede division of the nucleus. In one important respect the division of these nuclei differs from that of the amniotic nuclei. The cell does not divide immediately after the nucleus, and consequently a great number of cells are binucleate. Some even contain three nuclei. I have obtained no evidence whatever of cell division.

IV. Degenerative Changes.

Plate II. Figs. 14, 24-26 , Plate III. Figs. 28, 34.

The striking difference in the appearance of cells and nuclei, and the different manner of division of the nuclei, exhibited by serosæ of different ages, have frequently been referred to. Such changes, in part at least, I believe to be due to degeneration of the membranes, which, with the exception of the ovarian capsule, are temporary structures, soon to be cast off by the embryo. Hence it is not surprising to find them undergoing degeneration *in toto*. The degenerative changes are about equally well marked in all three membranes; but on account of the great size of cells and nuclei, the changes are most conspicuous in the serosa. If the membrane comes from a young embryo, the walls of the cells are unstainable, and therefore often difficult to make out. The nuclei have a vesicular appearance, with smooth, rounded contour, abundant karyoplasm, and scanty chromatic substance. For this reason the nuclei seldom stand out clearly from the cytoplasm in a stained preparation, often being no darker than the rest of the cell.

Serosæ from somewhat older embryos, while giving no sure signs of degeneration, have nuclei slightly different from those of the youngest membranes. The amount of chromatic substance appears to be larger. It is gathered into denser and more deeply staining masses, and the nucleoli become larger and more stainable (compare Figures 4 and 5, the former from an older membrane than the latter). Many nuclei at this stage become irregular in outline, and are more or less shrunken in appearance, changes which prepare the way for complete degeneration, found in membranes from the oldest embryos. The nucleus here becomes shrunken into a formless mass, which stains deeply and uniformly. This condition seems to be due almost wholly to loss of the karyoplasm, for the nuclear membrane is seen to be drawn closely over the much condensed chromatic substance. The uniformly staining effect, however, is generally believed to be produced by the solution of a part of the chromatin in the karyoplasm ; this is best seen in nuclei that have not completely degenerated, where the deeply stainable solid chromatin is immersed in the less stainable matrix. Not all the nuclei in a membrane are affected to the same degree by the degenerative change. This is shown in Figure 14, where the nuclei of cell *a*, and that of the cell farthest to the left, are more affected than any others. But in the oldest membranes almost every nucleus has undergone extreme degeneration.

It is an interesting fact, that even the most thoroughly degenerated membranes have numerous nuclei in all stages of division. The dividing nuclei have undergone the same degenerative alteration as the rest. It is impossible to state whether these nuclei had begun to divide *after* the regressive change, or had been overtaken by these changes while undergoing division; and it is equally impossible to say whether degeneration would have prevented the nuclei from completing their division. The division is essentially like that of younger nuclei, but often unsymmetrical.

Not all the degenerative changes are confined to the nuclei. The cells also give evidence of modification. Their walls become more distinct, not only because they are denser and thicker, but on account of their stainability with hæmatoxylin. The cytoplasm frequently has a reticulated structure, which is densest about the nucleus. In the oldest membranes, certain large groups of cells have nuclei surrounded by a narrow bright ring, and outside this a much broader halo of a radiating structure, which takes a deeper stain than the rest of the cytoplasm (see Fig. 34). The appearance of the whole is strikingly like that of the "attraction spheres" of ovarian and other cells, but in this case has certainly nothing to do with mitosis. If the cell contains two nuclei, or a dividing nucleus, each daughter nucleus is surrounded by a halo. In early stages of division, however, the elongated nucleus has a single halo. I am unable to account for these appearances; I do not regard them as attraction spheres, but rather as a result of degeneration. The attraction sphere should radiate from a centrosome; here it radiates from the nucleus as a centre. I may state, in passing, that my search for centrosomes in the serosa has been wholly unsuccessful. The pale ring is very generally present around nuclei that have undergone degeneration. It seems to have no intimate connection with the radiating zone, being frequently found where the latter is absent.

The life history of the serosa cells corresponds closely with that of certain cells in the Malpighian vessels of *Aphrophora spumaria* described by Carnoy ('85, p. 219). The cells at the two extremities of the tubes contain nuclei not greatly different from those of young serosæ, but the nuclei of the middle portion are irregular, jagged, and filled with amorphous chromatin. They therefore bear a strong resemblance to the degenerated nuclei of the serosa. Furthermore, the origin of the peculiar nuclei of the middle portion of the Malpighian vessel agrees closely with that of the degenerated nuclei of an old serosa. It is thus described by Carnoy (p. 220): "Sur les petites

larves on rencontre tous les intermédiaires entre les noyaux des extrémités et ceux du milieu. Peu à peu le boyau s'efface, le noyau lui-même se rétrécit et perd la régularité de ses contours à cause du plissement de sa membrane ; à la fin la nucléine ne forme plus à l'intérieur qu'une masse compacte et homogène, à peu près comme cela se présente dans la tête des spermatozoïdes." In both cases the degenerated nuclei are found in stages of division ; in both, the cytoplasmic reticulum is distinct only in old cells, and where these cells are binucleate it is dicentric, with filaments radiating from the nuclei. The *dicentricity* of the binucleate cells is a point to which Carnoy calls special attention (p. 229). He considers that here the radiating filaments of the cytoplasmic reticulum answer to the polar asters of karyokinesis, and that the nucleus has the function of a centrosome. The same reasoning would apply to the degenerated cells of the scorpion's serosa.

The regressive metamorphosis undergone by the epithelial cells of the ovarian capsule (Figs. 24–26) is very peculiar. Here, again, the cell walls are affected in the same way as in the serosa and amnion, for they are not distinctly seen until after the nuclei have degenerated. Nearly all of the epithelial cells of an old capsule have two nuclei, which are dissimilar in size and appearance (Figs. 24 and 25). The smaller takes a rather deep, uniform stain, almost as dark as that of the chromatin of the other. A nucleolus is always present, and frequently minute granules of chromatic substance. The uniformly staining character of the nucleus is doubtless produced by chromatic substance held in solution by the karyoplasm, a condition of common occurrence with degenerating nuclei. The larger nucleus (Figs. 24 and 25) takes only a slight stain, owing to the scantiness of its chromatic substance, which is present in the usual form of isolated granules and an imperfect network. By examination of a large number of cells, I found nuclear differentiation of every degree, beginning with nuclei almost alike in size and stainability (Fig. 24), then passing to examples of marked dissimilarity (Fig. 25), where the pale nucleus has become almost invisible, and the smaller deeply staining one has attained a very sharp, definite outline. As the pale nucleus becomes more and more shadowy, its shape becomes irregular. Near cells of this sort others can be found which contain only a single deeply staining nucleus (Fig. 26), the other having disappeared altogether. In case of trinucleate cells, I have invariably found two of them to be of the pale sort.

I am unable to offer any other explanation of these changes than that they are the result of degeneration or of decreased activity of the tissue.

But why one nucleus should become altered in one way, and the other in an entirely different manner, is difficult to say. A very similar differentiation of nuclei has been observed by Chun ('90) in the egg germs of a Siphonophore (*Stephanophys*). He found only *one* nucleus in the youngest germs, while the middle-sized and larger egg cells contained *two* of different size, the larger being pale, and the smaller staining intensely. The smaller nucleus moves to the periphery of the egg and is no longer visible when the latter is ripe. The larger nucleus persists as the germinative vesicle. In only one instance did he see a stage that showed that the smaller nucleus *budded out* of the larger. Chun compares the small, deeply staining nucleus to the "Stoffwechselkern" (macronucleus), and the pale one to the "Fortpflanzungskern" (micronucleus) of the ciliate Infusoria.

Summary.

1. The embryo of the scorpion is enveloped by three membranes, the ovarian capsule, the serosa, and the amnion.

2. The ovarian capsule is an enlargement of the ovarian tube; the serosa and amnion arise from the blastoderm of the egg.

3. Serosa and amnion are at first distinct, and joined to each other by minute fibres. These afterwards disappear, and the membranes coalesce.

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4. The serosa is composed of immense flat cells, very variable in size and shape. The cell walls are fibrillated.

5. The majority of the serosa cells have two large nuclei of equal size. There are rarely more than two.

6. The nuclei are disk-shaped, have a distinct nuclear membrane, and chromatin in the form of granules and filaments, the latter forming an indistinct reticulum. There are usually several nucleoli.

7. The cytoplasm of the serosa has a distinct reticular structure.

8. Nuclear division in the serosa is amitotic, and takes place by constriction, preceded by elongation of the nucleus. It is followed or accompanied by recession of the daughter nuclei, which remain for some time connected by a fine strand.

9. Constriction of the nucleus is usually accompanied by a longitudinal arrangement of some of the chromatic threads, radiating from the constricted part. The nucleoli are distributed about equally to the daughter nuclei.

10. Nuclear division may be followed by division of the cell, but not often immediately. The cell divides by the formation of a cell wall, either with or without constriction.

11. The binucleate condition of cells is independent of their size; but, in general, the size of the nucleus, or nuclei, is proportional to the size of the cell.

12. Elongated cells of the serosa are generally binucleate. The nuclei almost invariably lie in the long axis of the cell, near the ends.

13. A binucleate cell becomes trinucleate by division of *one* of its nuclei, and quadrinucleate by the division of *both*. Very rarely the division is tripartite, and the three nuclei are produced simultaneously from a single one.

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14. Division of the amniotic nuclei is also amitotic, but the constriction is supplemented by a septum at the equator of the elongated nucleus.

15. There is apparently no rearrangement of the chromatic substance. Nucleoli are apportioned equally to the daughter nuclei.

16. Division of the nucleus is quickly followed by division of the cell, so that binucleate cells are not common.

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17. The epithelium of the ovarian capsule is composed of small hexagonal or rectangular cells, which frequently contain two or more nuclei.

18. The nuclei are very similar to those of the amnion, but usually contain only one nucleolus.

19. Nuclear division is amitotic, and precisely like that of the amniotic nuclei. Each daughter nucleus contains one nucleolus.

20. No instance of cell division was observed.

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21. All three membranes undergo degeneration as the embryos approach maturity.

22. In the serosa the cytoplasmic reticulum becomes more distinct, and is seen to radiate from the nuclei. The cell-walls become stainable.

23. The chromatic substance of the nuclei becomes grouped into dense masses; the reticulum and nucleoli become more distinct. The outlines of the nuclei become irregular.

24. As degeneration proceeds, the cytoplasm frequently forms a halo of radial structure around the nucleus.

25. The nuclei finally become reduced to uniformly staining, irregular masses of chromatin, which has partly entered into solution. Such nuclei are found in all stages of division.

26. In binucleate cells of the ovarian epithelium the nuclei become dimorphic.

27. The chromatic substance of one of the nuclei enters into solution in the karyoplasm, and the nucleus becomes reduced in size.

28. The other nucleus loses its stainability, and increases in size. It finally disappears.

V. Discussion of Amitosis.

As long as karyokinesis was supposed to be a uniform process, all the complicated details of which were carried out with the greatest exactness and in the same sequence, wherever it occurred, no one sought to homologize it with the little known and far simpler "direct" division. The latter had, apparently, so restricted a range, and had received so little attention, that its very existence was denied; and it was generally anticipated that, in the few kinds of cells in which it was stated to occur, a better technique and more careful study would reveal mitotic phenomena. This opinion seemed to receive confirmation by the discovery of mitotic division in leucocytes and the Protozoa, thus carrying mitosis back to the simplest types of cells and to the lowest forms of life. The ascertainment of two facts has brought about a radical change in our views regarding amitosis: (1) the variability of karyokinesis, including, in some cases, the omission of apparently essential steps; and (2) the wide occurrence of amitosis, new instances of which are constantly coming to light in various parts of the Animal Kingdom. Inasmuch as it became necessary to recognize the existence of direct division, efforts were naturally made to find links connecting it with mitosis; the variability of both mitosis and amitosis seemed to lend strength to the theory which refers them to a single fundamental plan of division. In this scheme, amitosis is considered either as a primitive method from which mitosis was evolved, or else is looked upon as a degenerate form of mitosis, occurring in nuclei which, from their pathologic or exhausted condition, have lost the power of dividing by the more complicated process. By fixing epithelium of the salamander larva with osmic acid, then treating it with Müller's fluid, and finally staining with hæmatoxylin, Pfitzner ('86[a]) has shown conclusively that, even in cases of very perfect mitosis, the karyoplasm maintains its integrity, and divides

by a simple constriction, as in direct nuclear division. This fact has led Waldeyer ('88) to the conclusion that karyokinesis in based upon the simple scheme of division conceived by Remak. He says: "I would interpret the facts in such a way that we have to regard as the fundamental form the simple amitotic division, which is now proved for many cases; it always takes place where the nucleus either is poor in chromatin, or when it does not matter about strict bipartition of the chromatic material. Should the latter be required, then we shall find mitosis, since it is the most direct, most certain, and most simple manner in which an exact bipartition of chromatic substance is brought about."

It seems to me, however, that there are differences of so fundamental a character between mitosis and amitosis, as at present understood, that it is impossible to refer them to a single plan of division. Both, indeed, achieve the same result, — division of the nucleus, including its two constituents, chromatin and karyoplasm. In both cases, the karyoplasm divides by constriction. In amitosis, the chromatin undergoes little if any change in preparation for division; in mitosis it becomes consolidated into a limited number of thickened rods or loops (chromosomes), which arrange themselves in the plane of division ("mother star," "couronne équatoriale") and segment either longitudinally or transversely, the halves moving to opposite poles ("diaster"), and undergoing a reversed metamorphosis to form two daughter nuclei. If this were all there is to karyokinesis, — and in some cases the process is much simpler, — we might hope to find transitions between it and amitosis; for there are examples of amitosis in which the chromatic network undergoes changes during division, and it would be conceivable that the highly organized changes of the chromatic substance during mitosis were either evolved from them, or that they were a simplification of the more detailed changes. In mitosis, however, other structures besides chromosomes make their appearance, — the *centrosomes*, *attraction spheres*, and *spindle*. These structures are not known to take any part whatever in amitosis, and in this respect at least the two kinds of division are fundamentally different. The most recent workers upon karyokinesis agree in assigning to the spindle rays the function of *separating or dividing the chromosomes, and drawing* (*or pushing*) *the segments towards the poles.* The centrosomes are focal points towards which the spindle rays converge, and lie *entirely outside the nucleus.* The formation of the spindle has been carefully studied by many investigators of karyokinesis, and, while there are very divergent views as to its origin and mode of action, the most recent workers in this field (of whom

E. van Beneden, Boveri, and Watase may be mentioned) are agreed that the spindle arises *from the cytoplasm.* The same view with regard to the spindle in the mitosis of vegetable cells was expressed by Strasburger, Guignard, and other botanists.

The centrosome, as a converging point for the spindle fibres and polar rays, plays a most important part in karyokinesis, and, so far as known, none at all in amitosis. The centrosome has indeed been found by Flemming ('91) in leucocytes, which certainly divide amitotically; but there it is a single structure, and as Flemming's figures show, *takes no part in the amitotic division of the nucleus.* Whether it also remains passive during the *mitotic* division of leucocytes and in amitosis followed by division of the cell, is not known. It has been supposed by Carnoy ('85) that spindle rays were present in certain nuclei which divide amitotically, but this seems extremely doubtful, especially since they have no perceptible action on the chromatic substance. I believe it can be shown in every case of amitosis known, that the division of the chromatin is accomplished *independently of chromosomes, spindle rays, or any other visible influence outside of the nucleus.*

The persistence of the nuclear membrane in amitosis, and its disappearance in mitosis, were formerly considered points of distinction between the two kinds of division; but, as is well known, more recent studies have shown that the membrane persists in many cases of undoubted karyokinesis, especially among the Arthropods (Carnoy, '85) and Protozoa (Gruber, '83, R. Hertwig, '84, Pfitzner, '86[b], and Schewiakoff, '88). Its presence seems to offer no obstacle to the karyokinetic changes, and Watase ('91) has pointed out that it need not prevent the formation of an extra-nuclear spindle, the rays of which may penetrate the membrane. In the nuclei of *Opalina ranarum,* and in the micronuclei of Infusoria generally, where, according to all observers, the nuclear membrane persists, the mitotic division is accompanied by constriction; but the fact that constriction is here *visible* may be considered as in some measure a *result* of the persistence of the membrane, thereby making evident the outline of the karyoplasm. Yet constriction does not always take place when the membrane persists, for in the spermatic cells of *Pagurus striatus,* figured by Carnoy ('85, Plate VII. Fig. 244), the nuclear membrane is visible at all stages, and gives no evidence of constriction.

The modification of the chromatic substance into chromosomes is usually the most conspicuous feature of karyokinesis, and in most cases serves to distinguish mitotic nuclei from any of the amitotic ones. The

chromosomes invariably include *all* the stainable substance of the nucleus, so that the presence of nucleoli in a nucleus undergoing constriction may be taken as perhaps the strongest evidence of direct division. The behavior of nucleoli in amitosis is of peculiar interest. Where there is a single nucleolus, it constricts previous to the constriction of the nucleus, according thus with the Remakian scheme. The division of the nucleolus, however, has rarely been observed. It was first described, I believe, by F. E. Schulze ('75), in the division of *Amœba polypodia;* has since been figured by Carnoy ('85, Plate I. Figs. 10, 12, 13) for various amitotically dividing Arthropod cells, and by Hoyer ('90) for the intestinal epithelium of *Rhabdonema nigrovenosum.* A peculiar modification of the nucleolus, and its division into four segments previous to the constriction of the nucleus, was observed by Platner ('89, pp. 145–149) in the Malpighian vessels of *Dytiscus marginalis.* It is extremely probable that, whenever the nucleolus is a single and definitely organized structure, it always divides previously to or during constriction of the nucleus. Where there are several small nucleoli, they may indeed arrange themselves so as to be equally apportioned to the daughter nuclei; but they are not known to divide, as the chromosomes in mitosis do.

Amitotic division, even more than karyokinesis, is variable in its phenomena. It takes place by constriction, by formation of division planes, by gemmation, and by enlargement of one or more perforations (Arnold, '88, Flemming, '89). It is either simple or multiple, and it may or may not be accompanied by division of the cell. The resulting nuclei may be equal or unequal. Amitosis occurs throughout both the Animal and Vegetable Kingdoms; but as far as animals are concerned, it is far the most frequent among *unicellular organisms, amœboid cells (leucocytes), and epithelial tissues.* There seem to be no authentic instances of it in connective tissues (except possibly the fat-cells of Arthropods, described by Carnoy), none in nervous tissue, and but one or two in muscle fibres (Carnoy, '85, p. 221). Not only the nuclei of fixed tissues divide by the direct method, but also those of nascent tissues, at least among the Arthropods. Direct division is, however, of rare occurrence in the embryo. I believe there are only two authentic instances of it, — that discovered by Carnoy in the ventral plate of an embryo of *Hydrophilus piceus* ('85, p. 224, Plate I. Fig. 11), and that found by Wheeler ('89, p. 313) in the formation of the blastoderm of *Blatta germanica,* where no instance of mitosis was detected. The embryonal membranes of the scorpion I do not include under this head, because they are temporary structures forming no vital part of the embryo.

Among the Metazoa, epithelial tissues offer by far the greatest number and the most interesting cases of amitosis. Furthermore, as Ziegler ('91) has very recently shown, *epithelial cells of unusual size, with some peculiar functional activity (generally secretion) are most apt to exhibit this method of division.* Cell division has seldom been observed to follow amitosis in such large cells, which therefore become multinucleate. Other epithelial cells which frequently furnish instances of amitosis are those *which are near the end of their functional activity.* Cells of the *outer* layer of a stratified epithelium sometimes divide amitotically, while those of the *deeper* (and therefore *younger*) layers of the same epithelium divide by mitosis. A good instance of this was recently described by Dogiel ('90) in the epithelium of the bladder of Mammals. The nuclei of the large epithelial cells lining the intestine of Arthropods very commonly divide by amitosis, as was found by Frenzel ('85) in the midgut of *Astacus* and *Maja;* by Carnoy ('85) in the intestinal epithelium of Isopods; and by Faussek ('87) in the digestive tract of a Cricket (*Eremobia muricata*) and in the larva of *Æschna.* The intestinal epithelium in all Arthropods has an important secretory function. Cells whose function is excretory likewise exhibit amitotic division of the nucleus, as in the Malpighian vessels of Insects. The occurrence of amitosis in glandular and excretory epithelium is readily explainable on Chun's hypothesis, for the functional activities of such cells are peculiarly intense, and it is easy to see that a distribution of nuclear material in the cytoplasm is of advantage to the cell. The occurrence of nuclei of unusual size (as compared with the nuclei of other cells of the same animal) seems to me likewise referable to the peculiar needs of the cytoplasm in these cells.

Cases of amitosis peculiarly difficult of explanation are those presented by the germinal epithelium of the testis. So many observers have reported direct division in sperm mother-cells, that there seems no reasonable doubt of its occurrence. It has been suggested that the cells which divide amitotically never produce spermatozoa, but merely serve to secrete a fluid. This explanation, however, will not serve in the case of certain Isopods (*Oniscus asellus* and *Idotea* sp.) in the testes of which Carnoy ('85, p. 222) found amitosis the prevailing type of division, and mitosis of very rare occurrence. Direct division is found more or less frequently in the testicular cells of many other Crustacea, as the extensive work of Gilson ('84–87), and the investigations of Sabatier ('85) show, and occasionally in the other groups of the Arthropods. Among Vermes, it was found by Lee ('87) in Nemertians, and

by Löwenthal ('89) in a Nematode (*Oxyuris ambigua*). It need hardly be said that amitosis in sexual cells is unexplained by any hypothesis yet offered regarding the biological significance of this type of division, and further investigations on this point are absolutely necessary before we can form any general opinion in regard to it.

In the maturation and segmentation of the ovum no instance of direct division is known, and it is here that karyokinesis is exhibited in its most complete form. The well known observations of Boveri ('87) on the segmentation of the egg of *Ascaris megalocephala* are of special interest on this point. He found a modification of the chromatic threads as early as the two-blastomere stage, one of them (cell A) retaining the four chromosomes characteristic of the nucleus after fertilization, the other (cell B) undergoing a reduction of its chromosomes into the form of granules. The two blastomeres arising by division of cell A undergo the same differentiation, the nucleus of one (cell A^1) retaining the chromatic loops, the other (cell A^2) undergoing reduction, so that in the four-cell stage only one nucleus has retained its chromatic loops. The systematic reduction of chromosomes was observed up to the 64-cell stage. The important deduction Boveri makes from these facts is, that the cells retaining their ancestral nuclear characters are the *Anlage* of the sexual cells of the developing animal, and that the cells whose nuclei undergo a modification of the chromosomes are all somatic cells. In accordance with this hypothesis, the division of both male and female sexual cells ought always to be karyokinetic, and of a somewhat different type from the karyokinesis of the somatic cells of the same animal. The latter statement, indeed, holds true for the testicular cells of the salamander, as was discovered by Flemming ('87). It also appears from the work of Carnoy, that in the post-embryonic life of Arthropods mitotic division is of rare occurrence in the tissue cells, but is of constant occurrence in the reproductive cells of the same forms.

As has already been stated (p. 147), attempts have been made to find a morphological connection between karyokinesis and direct division, and thus to solve the puzzling question of the relations they bear to each other. Carnoy ('85, p. 398) believes he has found transitions between them in the division of the numerous nuclei of *Opalina ranarum*. Some of these show a distinct spindle, others none ; in both cases the nuclear membrane persists, and division is accomplished by constriction. Pfitzner ('86[b]), however, found only mitosis in *O. ranarum*. Carnoy has also seen transitional forms of division in the spermatic cells of

Pagurus striatus, and *P. callidus* (Planche VII. Figs. 244, 245). A nuclear plate is here formed, both in perfect mitosis and in degenerated mitosis; but in the former instance a spindle is formed, and the chromosomes segment individually, while in the latter the plate divides *in toto* by constriction, without the help of a spindle. This modified type of mitosis, if we may so regard it, Carnoy considered as the result of degradation (pp. 316, 317), inasmuch as it appeared only in *old* sperm mother-cells after spermatozoa had become numerous in the testis. This accords with the earlier view that direct division is concomitant with senescence of the nuclei, based especially upon nuclear division in plants (Schmitz, '79, Johow, '81). I have regarded this as a possible explanation of the occurrence of amitotic division in the embryonal envelopes of the scorpion, for these tissues are temporary structures which obviously are near the end of their functional activity. This explanation, however, will not fit all cases; for instance, the occurrence of amitosis in embryonic cells, and its prevalence in the testicular cells of some Isopods, already mentioned.

The hypothesis advanced by Chun seems to throw light upon many of the cases of amitotic division which are referable to a sort of *budding* or *branching* of the nucleus, carried to such a point that the buds or branches become constricted off as separate nuclear elements. These cases are, of course, not to be confounded with a disintegration of the nucleus, such as takes place in the macronucleus of Infusoria after conjugation, and sometimes in the degeneration of tissues. The distribution or extension of nuclear substance in the cytoplasm, whereby the surface of the nucleus is increased, is an event of frequent occurrence. It is seen in the many forms of lobed nuclei, such as those of the ovarian capsules of Amphibia (see Flemming, '82), and in those of leucocytes; in hollow or perforated nuclei (giant cells); in branched nuclei (spinning glands and Malpighian vessels of Lepidoptera); and in the band-shaped and moniliform nuclei of many Infusoria. These peculiar shapes are evidently produced by the *activity of the nucleus itself*, probably correlated with a special function of the cytoplasm. From the deeply incised lobation or band-shape of such nuclei it is an easy step to the formation of separate smaller nuclei by the deepening of a constriction already formed. Such daughter nuclei will as a rule be irregular in shape and unequal in size; but if their production subserves a definite and important function, we should expect that in some cases their formation would become a regular process, governed by definite laws. It is possible that the more symmetrical kinds of direct division

are to be explained in this way, and such an explanation seems to apply well, as suggested on a preceding page, in the case of the scorpion's serosa. Division of the cell does not follow as a rule, and upon this fact Chun lays stress. But, so far as we know, there is nothing to exclude the *subsequent* occurrence of cell division, and it is even probable that cell division is induced by the presence of more than one nucleus. This I take to be the case in the scorpion's serosa, where I believe the division of the cell is due in part to the dicentricity set up in the cytoplasm by the division of the nucleus.

The study of nuclear division among the Protozoa seems likely to throw much light upon the relations of amitosis to mitosis, for there can be little doubt but that this group presents the most primitive types of nuclear division. So far as known, the very lowest forms of animal cells (*Amœbæ*) always divide by the direct method, as the study of *Amœba polypodia* by F. E. Schulze ('75), and of *Pelomyxa villosa*, *Amœba secunda*, and *A. proteus* by Gruber ('83 and '85), has shown. The division of the nucleus of *Amœba proteus* takes place by a sharp equatorial cleft, passing through the large, centrally placed nucleolus, and dividing that and the peripheral zone of chromatin into two exactly equal halves, which afterwards move apart. This is regarded by Gruber ('83, p. 385) as a simple type of karyokinesis, because an exact division of the chromatin is accomplished. No kinetic change of the chromatic substance is necessary to bring this about, hence none occurs. It seems to me that the absence of centrosomes and a spindle effectually separates this type of division from true karyokinesis, and until these are discovered, the nuclear division of *Amœba proteus* must be relegated to amitosis. The presence of so perfect a type of karyokinesis as that found in *Euglypha alveolata*, worked out so completely by Schewiakoff ('88), is strong evidence against the hypothesis that karyokinesis was gradually evolved from direct division. For here, among the lowest forms of animal life, we have nuclei dividing both by a simple constriction, and by the most highly developed kinetic changes.

Nuclear division among the Infusoria is of special interest, for we regularly find in the same individual nuclei very different in structure and function, — macro- and micronuclei. The former divide directly, the latter by karyokinesis. Apparent exceptions are seen in *Spirochona gemmipara*, where, according to R. Hertwig ('77) the macronucleus divides by karyokinesis; and in *Opalina ranarum*, studied most carefully by Pfitzner ('86[b]). As only one kind of nucleus is found in Opalina, it is probable, as Bütschli suggests ('88, p. 1500), that these are of

the micronuclear type, inasmuch as the division is in all essential respects like that of micronuclei, and in the resting state the nuclei bear no resemblance to macronuclei. The direct division of macronuclei is often accompanied by a longitudinal arrangement of the chromatic filaments, resembling that found in the scorpion's serosa (see Figs. 6, 7, 8). It seems to me that Carnoy is wrong in speaking of these longitudinal filaments as a "spindle," for it has never been shown that they converge to the poles of the nucleus, and frequently they can be resolved into granules, which is never the case with spindle fibres. Their resemblance to the spindle of karyokinesis is deceptive. From their behavior with stains, I regard them as consisting of chromatin, and Bütschli ('88, p. 1526) speaks of this stage of the macronucleus as the "Knäuelstadium," implying that the parallel filaments are chromatic threads.

Among the Vertebrates, amitosis is unusual, and where it exists karyokinesis is generally found to occur in cells of the same kind. It is almost confined to cells *which do not form fixed tissues*, as leucocytes of all kinds, and "giant cells," especially those of the red marrow. It also occurs in testicular cells of Vertebrates. In leucocytes, according to all observers, the nuclear division takes place by constriction, and is frequently accompanied by division of the cytoplasm (Ranvier, '75; Flemming, '82, p. 344; Arnold, '87). But, as the recent work of Flemming ('91) and others shows beyond a doubt, leucocytes also divide by karyokinesis. It is difficult to say whether there is more than a single kind of leucocyte, one dividing directly, the other indirectly, or whether cells of the same kind divide in two different ways. In case of giant cells, it has been shown by Arnold ('84), Denys ('86), Demarbaix ('89), and others, that division occurs both directly and by multiple karyokinesis. Both kinds of division are followed by division of the cytoplasm, leading to the formation of a brood of daughter cells within the mother cell.

After going over the literature of amitosis, taking especial note of the manner of its occurrence and distribution in the Animal Kingdom, I have become convinced that it is not derived from mitosis, and, on the other hand, is not the forerunner of the more complicated process. I consider it another type of division altogether, which, along with karyokinesis, has been transmitted from the simplest forms of life to the most highly organized. While apparently every kind of nucleus may, at some stage of its existence, divide by karyokinesis, many afterwards exchange this type of division for the simpler process. The special conditions which evoke the exchange are very imperfectly understood,

and no hypothesis has yet been offered that will explain all the known instances. Some of the hypotheses that have been suggested I have already dwelt upon at length; others, as *scantiness* of chromatin, and even its *entire absence* in the nucleus (Löwit, '90), seem to me still more inadequate.

One fact in favor of the independence of the two types of division is the *sudden change from mitosis to amitosis, without any visible intermediate stages.* Phylogenetically, this is seen in the abrupt transition from the amitotic division of *Amœbæ* to the very perfect karyokinesis of the nearly related *Euglypha.* Ontogenetically, of course, the exchange is far more abrupt. In the conjugation of Infusoria, all divisions of the micronucleus are undoubtedly mitotic, while the *first* (after conjugation) and all subsequent divisions of the macronucleus, *itself formed from modified micronuclei,* are by direct division. Again, the amitosis of the blastodermic nuclei of *Blatta* (Wheeler, '89) is an abrupt change from the perfect mitosis of segmentation. Other instances are the sudden change from mitosis to amitosis in the layers of stratified epithelium, and in the generations of spermatic cells.

Another fact in favor of my view is the *almost universal distribution of amitosis, and its occurrence in many kinds of cells with widely different functions.* It seems more reasonable to suppose that a process so widely extended is *inherited,* and exists potentially in all cells, rather than to look upon it as independently assumed in a multitude of special cases. The latter supposition is opposed to all we know of the transmission of fundamental characters.

While it is evident that both mitosis and amitosis appeared at a very early period of organic life, it is impossible to say which appeared first. But, on *a priori* grounds, we may conclude that the simpler type preceded the more complex.

CAMBRIDGE, September 28, 1891.

It was not until this paper had gone to press that I had access to the recent communications on amitosis by Flemming ('91[a]), Löwit ('91), Verson ('91), Frenzel ('91), and O. vom Rath ('91). In his review of recent work on cell division, Flemming says (p. 139): "Es ist also nicht nur als feststehend anzusehen, dass Amitose vorkommt, sondern auch, dass sie in normal lebenden Geweben vorkommt, und dass sie zur

Zellenvermehrung führen *kann*." When, however, both mitosis and amitosis occur in the same tissue, he considers it probable that only the former is the *normal* method of regeneration and of growth.

The brief papers by Löwit, Verson, and Frenzel are replies to Ziegler's ('91) recent article on amitosis, and contain little that is new. Verson describes briefly the early stages in the spermatogenesis of the silkworm (*Bombyx mori*). He states that the spermatocytes originate from a single large nucleus ("Riesenkern"), which divides repeatedly and unequally by amitosis. The small daughter nuclei thus produced *divide by mitosis*, and at length form the spermatocytes. Frenzel adduces instances of amitosis in the intestinal epithelium of Crustacea and Insects which do not fall within Ziegler's generalizations.

Vom Rath's paper is a valuable contribution to our scanty knowledge of the occurrence of amitosis in spermatogenesis. He shows very conclusively that, in the testis of the crayfish, amitosis does not occur in the generations of sperm-forming cells, but only in abortive nuclei ("Randkerne"), which soon degenerate into an amorphous mass. If such a fate could be established for all amitotically dividing nuclei in the testes of animals, it would be much easier to form a logical estimate of amitosis.

BIBLIOGRAPHY.

Arnold, J.

'84. Ueber Kerntheilung und vielkernige Zellen. Virchow's Arch. f. pathol. Anat., Bd. XCVIII. p. 501.

'87. Ueber Theilungsvorgänge an den Wanderzellen, ihre progressiven und regressiven Metamorphosen. Arch. f. mikr. Anat., Bd. XXX. p. 205.

'88. Weitere Mittheilungen über Kern- und Zelltheilungen in der Milz., etc. Arch. f. mikr. Anat., Bd. XXXI. p. 541.

Blochmann, F.

'85. Ueber directe Kerntheilung in der Embryonal-hülle der Scorpione. Morph. Jahrb., Bd. X. p. 480.

Boveri, T.

'87. Ueber Differenzirung der Zellkerne während der Furchung des Eies von Ascaris megalocephala. Anat. Anz., Jahrg. II. p. 688.

Bütschli, O.

'76. Studien über die ersten Entwickelungs-vorgänge der Eizelle, die Zelltheilung, und die Conjugation der Infusorien. Abhandl. Senckenberg. Naturf. Gesellsch., Bd. X. p. 213.

'88. Protozoa. Abth. III. Infusoria und System der Radiolaria. Bronn's Classen u. Ord. des Thier-Reichs, Bd. I. Abth. 3, p. 1098.

Carnoy, J. B.

'85. La Cytodiérèse chez les Arthropodes. La Cellule, Tom. I. p. 191.

Chun, C.

'90. Ueber die Bedeutung der direkten Kernteilung. Sitzungsber. Physik-ökonom. Gesellsch. Königsberg i. Pr., Jahrg. 31, 6 pp.

Demarbaix, H.

'89. Division et dégénérescence des cellules géantes de la moëlle des os. La Cellule, Tom. V. p. 27.

Denys, J.

'86. La Cytodiérèse des cellules géantes et des petites cellules incolores de la moëlle des os. La Cellule, Tom. II. p. 245.

Dogiel, A. S.

'90. Zur Frage über das Epithel der Harnblase. Arch. f. mikr. Anat., Bd. XXXV. p. 389.

Faussek, V.

'87. Beiträge zur Histologie des Darmkanals der Insekten. Zeitschr. f. wiss. Zool., Bd. XLV. p. 694.

Flemming, W.

'82. Zellsubstanz, Kern- und Zelltheilung. Leipzig, F. C. W. Vogel.

'87. Neue Beiträge zur Kenntniss der Zelle. Arch f. mikr. Anat., Bd. XXIX. p. 389.

'89. Amitotische Kerntheilung im Blasenepithel des Salamanders. Arch. f. mikr. Anat., Bd. XXXIV. p. 437.

'91. Ueber Theilung und Kernformen bei Leucocyten, und über deren Attractionssphären. Arch f. mikr. Anat., Bd XXXVII. p. 249.

'91a. Ueber Zellteilung. Verhandl. Anat. Gesellsch., 5. Versamm., p. 125.

Frenzel, J.

'85. Ueber den Darmkanal der Crustaceen, nebst Bemerkungen zur Epithelregeneration Arch. f. mikr. Anat., Bd. XXV. p. 137.

'91. Zur Bedeutung der amitotischen (direkten) Kernteilung. Biol. Centralb , Bd. XI. p. 558.

Gehuchten, A. van.

'89. L'Axe organique du noyau. La Cellule, Tom. V. p. 177.

Gilson, G.

'84–'87. Étude comparée de la spermatogénèse chez les Arthropodes. La Cellule, Tom. I. p. 11; Tom. II. p. 83; Tom IV p. 1.

Göppert, E.

'91. Kerntheilung durch indirekte Fragmentirung in der lymphatischen Randschicht der Salamandrinenleber Arch. f. mikr. Anat., Bd. XXXVII. p 375.

Gruber, A.

'83. Ueber Kerntheilungsvorgänge bei einigen Protozoen. Zeitschr. f. wiss. Zool., Bd. XXXVIII. p. 372.

'85. Studien über Amöben. Zeitschr. f. wiss. Zool., Bd. XLI. p. 186.

Hamann, O.

'90. Monographie der Acanthocephalen. Teil I. Jena. Zeitschr., Bd. XXV. p. 113.

Hertwig, R.

'77. Ueber den Bau und die Entwickelung der Spirochona gemmipara. Jena. Zeitschr., Bd. XI. p. 149

'84. Ueber die Kerntheilung bei Actinosphaerium eichhorni. Jena. Zeitschr., Bd XVII. p. 490.

Hoyer. H.

'90. Ueber ein für das Studium der direkten Kerntheilung vorzüglich geeignetes Objekt. Anat. Anz , Jahrg. V. p. 26.

Johow, F

'81. Die Zellkerne von Chara foetida. Bot. Zeit., Jahrg. XXXIX. Nos. 45, 46.

Kowalevsky, A., und M. Schulgin.

'86. Zur Entwicklungsgeschichte des Skorpions (Androctonus ornatus). Biol. Centralb , Bd. VI. p. 525.

Kükenthal, W.
'85. Ueber die lymphoid Zellen der Anneliden. Jena. Zeitschr., Bd. XVIII. p. 319.

Laurie, M.
'90. Embryology of a Scorpion (Euscorpius Italicus). Quart. Jour. Micr. Sci., Vol. XXXI. p. 105.

Lee, A. B.
'87. La Spermatogénèse chez les Némertiens. Recueil zool. Suisse, Tom. IV. p. 409.

Löwenthal, N.
'89. Ueber die Spermatogénèse von Oxyuris ambigua. Internat. Monatsschr. f. Anat. u. Physiol., Bd. VI. p. 364.

Löwit, M.
'90. Ueber Amitose (directe Theilung). Centralb. f. allgem. Pathol. u. pathol Anat., Bd. I. p. 281.
'91. Ueber amitotische Kernteilung. Biol. Centralb., Bd. XI. p. 513.

Metschnikoff, E.
'71. Embryologie des Skorpions. Zeitschr. f. wiss. Zool., Bd. XXI. p. 204.

Overlach, M.
'85. Die pseudomenstruierende Mucosa uteri nach acuter Phosphorvergiftung. Arch. f. mikr. Anat , Bd. XXV. p. 191.

Pfitzner, W.
86[a]. Zur morphologischen Bedeutung des Zellkerns. Morph. Jahrb., Bd. XI. p. 54.
'86[b]. Zur Kenntniss des Kerntheilung bei den Protozoen. Morph. Jahrb., Bd. XI. p. 454.

Platner, G.
'89. Beiträge zur Kenntniss der Zelle und ihre Theilungserscheinungen, I–III. Arch. f. mikr. Anat., Bd. XXXIII p. 125.

Rabl, C.
'85. Ueber Zelltheilung Morph. Jahrb., Bd. X. p. 214.
'89. Ueber Zelltheilung. Anat Anz., Jahrg. IV. p. 21.

Ranvier, L.
'75 Recherches sur les éléments du sang. Travaux Lab. d'Histol., p. 1.

Rath, O. vom
'91. Ueber die Bedeutung der amitotischen Kerntheilung im Hoden. Zool. Anz , XIV. Jahrg. Nos. 373, 374, 375.

Sabatier, A.
'85. Sur la spermatogénèse des Crustacés décapodes. Compt. Rend., Tom. C. p. 391

Schewiakoff, W.
'88. Ueber die karyokinetische Kerntheilung der Euglypha alveolata. Morph Jahrb., Bd. XIII p. 193.

Schmitz, F.
'79. Beobachtungen über die vielkernigen Zellen der Siphonocladiaceen. Halle.

Schulze, F. E.
'75. Rhizopoden-studien, V. Arch. f. mikr. Anat., Bd. XI. p. 583.

Verson, E.
'91. Zur Beurteilung der amitotischen Kernteilung. Biol. Centralb., Bd. XI. p. 556.

Waldeyer, W.
'88. Ueber Karyokinese und ihre Beziehungen zu den Befruchtungsvorgängen. Arch. f. mikr. Anat., Bd. XXXII. p. 1. [Transl. Quart. Jour. Micr. Sci., Vol. XXX. p. 159]

Watase, S.
'91. Studies on Cephalopods. I. Cleavage of the Ovum. Jour. Morph., Vol. IV. p. 247.

Wheeler, W. M.
'89. The Embryology of Blatta germanica and Doryphora decemlineata. Jour. Morph., Vol. III. p. 291.

Woodworth, W. M.
'91. On the Structure of Phagocata gracilis, Leidy. Bull. Mus. Comp. Zoöl., Vol. XXI. p. 1.

Ziegler, H. E.
'87. Die Entstehung des Blutes bei Knochenfischembryonen. Arch. f. mikr. Anat., Bd. XXX. p. 596.
'91. Die biologische Bedeutung der amitotischen (direkten) Kernteilung im Tierreich. Biol. Centralb., Bd. XI. p. 372.

EXPLANATION OF FIGURES.

All figures are from drawings made with the aid of an Abbé camera.

PLATE I.

Fig. 1. Five cells of the serosa, two of them covered by the amnion, which is omitted from the rest of the figure for the sake of clearness. *am.*, amnion; *sr.*, serosa. × 150.

Fig. 2. Section through the embryonal membranes and ovarian capsule. The fibrous appearance of the ovarian capsule is due to the presence of muscle fibres and connective tissue. The boundary line between amnion and serosa is visible only in the vicinity of the amniotic nuclei. *e'th. fol.*, epithelium of ovarian capsule (when the plates were engraved I still took this to be the follicular epithelium, hence the error in the abbreviation); *nl. fol.*, nucleus of capsular epithelium; *nl. sr.*, nucleus of serosa; *nl. am.*, nucleus of amnion. × 630.

Figs. 3–15 are all from the *serosa*.

Fig. 3. Very small, binucleate cell. × 130.

Figs. 4–10. Nuclei at different stages of division. *vac.*, vacuole; *x*, new nuclear wall within the old one. × 550.

Fig. 11. Two cells produced by division of a binucleate cell. × 130.

Fig. 12. Cell from the serosa of a young embryo, with dividing nucleus; the axis of elongation corresponds with the *short* axis of the cell. × 130.

Fig. 13. Cell from serosa of a young embryo, with nucleus unequally divided and daughter nuclei eccentric in position. × 130.

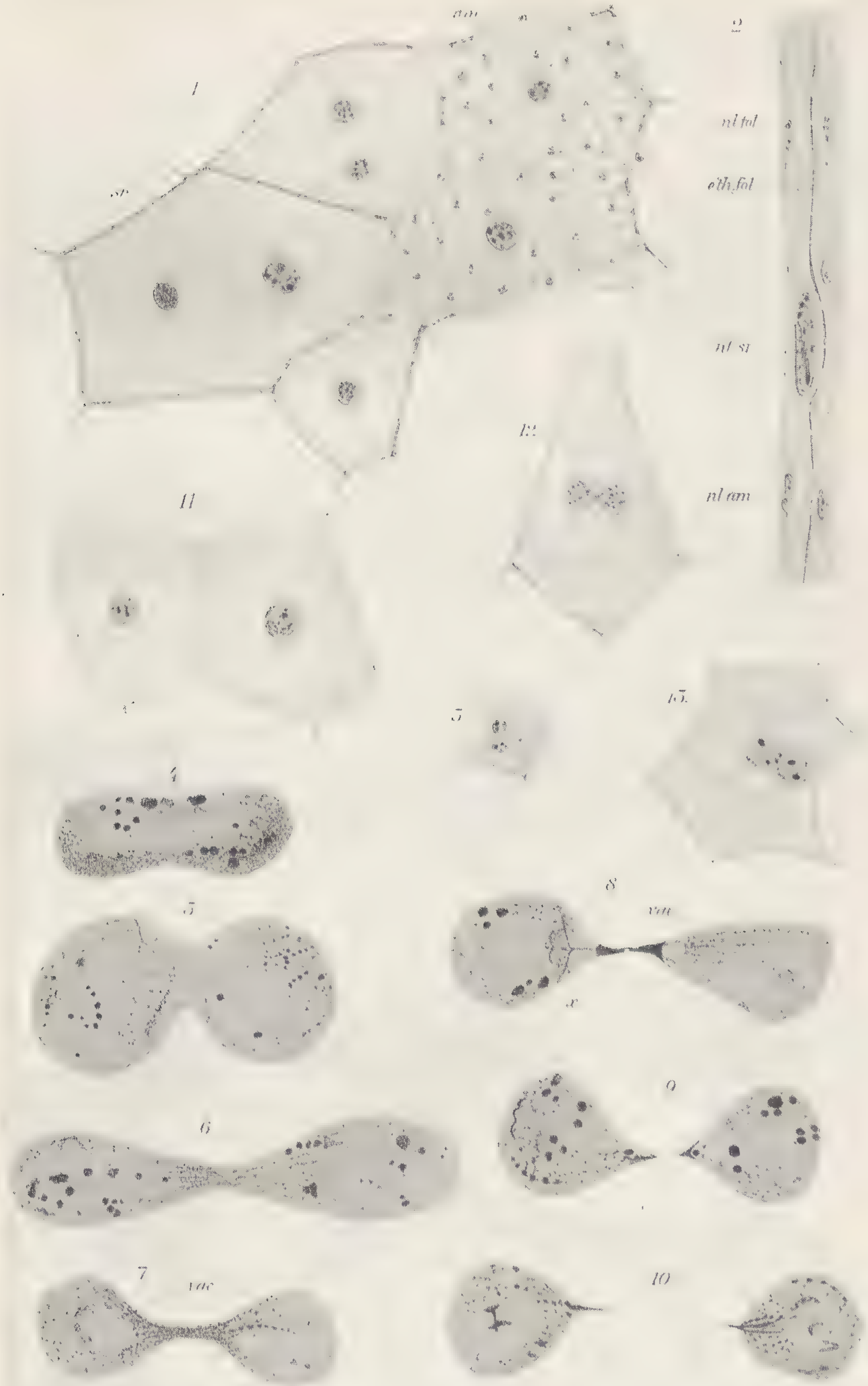
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PLATE II.

Fig. 14. Piece of the serosa from an advanced embryo, with four adjacent trinucleate cells (1, 2, 3, 4); nuclei of cell *a* and the large cell farthest to left have undergone degeneration. × 90.

Fig. 15. Three cells of the serosa from an old embryo to show recession of daughter nuclei towards the ends of the cells. × 90.

Figs. 16–20 are from the *amnion*.

Figs. 16–19. Stages in the division of amniotic nuclei. In Figure 18 three stages are shown, *a*, *b*, *c*. × 800.

Fig. 20. Two amniotic cells, apparently formed by recent division. × 375.

Figs. 21–26 are from the *capsular epithelium*.

Figs. 21–23. Cells showing successive stages of nuclear division. × 800.

Figs. 24–26. Cells to show the degeneration of nuclei. In Figure 24 the nuclei are but slightly differentiated; in Figure 25 the pale nucleus has become much larger and very faint; in Figure 26 it has disappeared altogether. × 800.

H. P. J. del.

PLATE III.

Figs. 27–34 are all from the *serosa*.

Fig. 27. A cell undergoing division by formation of a cell plate. The daughter nuclei are still united by a connecting thread. The dotted line on the left indicates the edge of the fragment of membrane in which this cell occurs. From the serosa of an advanced embryo. × 304.

Fig. 28. A cell divided by constriction, without the formation of a cell plate. The nuclei have undergone degeneration. From the serosa of an advanced embryo. × 150.

Fig. 29. A cell, the nucleus of which has undergone tripartite division. From an old serosa. × 150.

Fig. 30. Nucleus of the same, more highly magnified. The chromatin is grouped in granular masses. Two of the daughter nuclei are still united by strands of the nuclear membrane. × 630.

Figs. 31–32. Constricted nuclei from a young serosa. One of the daughter nuclei of each is larger than its mate, and has itself become elongated and constricted. × 304.

Fig. 33. Quadrinucleate cell. The upper of the two original nuclei has divided in a longitudinal, the lower in a transverse plane. Nucleus *a* still shows a remnant of the connecting thread, and nucleus *b* retains the conical form it had in division. Both nuclei have rotated 90° from the plane of elongation. × 304.

Fig. 34. Cell from the serosa of a far advanced embryo. The nuclei have undergone extreme degeneration. Each nucleus is surrounded by a bright ring, outside of which is a broad zone of a radiate structure, more stainable than the rest of the cytoplasm. × 150.

No. 4. — *A Fourth Supplement to the Fifth Volume of the Terrestrial Air-Breathing Mollusks of the United States and Adjacent Territories.* By W. G. BINNEY.[1]

THE following pages are believed to contain all that has been added to our knowledge of the subject prior to date.

Students are requested to note that in the Third Supplement, p. 214, the figures of *Arionta Diabloensis* and *Bridgesi* are reversed. On p. 225, Explanation of Plate VII., the references E and F are reversed: on p. 226, Explanation of Plate XI., Figures D and G are reversed.

BURLINGTON, NEW JERSEY, July 1, 1891.

Glandina decussata, DESH.

Plate I. Fig 4.

Under the name of *decussata*, specimens are found in most collections which can hardly be referred to that species. I have figured one of them, and its dentition has already been described and figured in my Third Supplement. The shell is readily recognized by its more cylindrical form. Should it prove distinct from *decussata*, I would suggest for it the specific name of *Singleyana*. I received it from Bexar County, Texas, collected by Mr. Wetherby.

Selenites Vancouverensis, LEA, var Keepi, HEMPHILL.

Plate II. Fig. 5.

Shell umbilicated, greatly depressed, thin, smooth, shining, transparent, scarcely marked by the delicate wrinkles; very light horn-color; whorls over four, somewhat flattened above and beneath, and scarcely descending at the aperture; spire

[1] The Terrestrial Air-Breathing Mollusks of the United States and the Adjacent Territories of North America, described and illustrated by Amos Binney. Edited by A. A. Gould. Boston, Little and Brown, Vols. I., II., 1851; Vol. III., 1857. Vol. IV., by W. G. Binney, New York, B. Westermann, 1859 (from Boston Journ. Nat. Hist.). Vol. V., forming Bull. Mus. Comp. Zoöl., Vol. IV., 1878. Supplement to same, in same, Vol. IX. No. 8, 1883. Second Supplement, in same, Vol. XIII. No. 2, 1886. Third Supplement, in same, Vol. XIX. No. 4, May, 1890.

flat, not rising above the body whorl; suture well impressed; umbilicus moderately large, exhibiting most of the volutions; aperture transversely subcircular, wider than high; lip simple, thickened, sinuous above, very slightly reflected at the base, ends scarcely approached Width $\frac{5}{16}$ inch, height $\frac{2}{16}$ inch.

Hills near Oakland, California. One specimen only.

This rare and interesting little shell I collected some years ago. It is a perfect miniature form, in every respect, of *S. Vancouverensis.* I regard it as an extremely small variety of that so called species. It is about the size of the variety of *S. Duranti*, lately described as *S. cœlatus*, Mazyck, but differs very materially in form, sculptnre, and the general texture of the shell. It differs from var. *Catalinensis* in being more robust, larger, and has a smaller umbilicus. I dedicate this pretty little shell to Prof. Josiah Keep, of Mills College, California, who has done so much through his interesting little book to stimulate the study of West Coast shells.

The above is Mr. Hemphill's description, from "The Nautilus," Vol. IV. p. 42, 1890. My figure is drawn from an authentic specimen.

Selenites Vancouverensis, var. hybridus, Hemphill.

Shell broadly umbilicated, depressed, slightly convex above, surface shining, polished, of a dark yellowish green color, lines of growth coarse, rib-like and regular on the spire, finer and more irregular on the body whorl, crossed by fine revolving lines that become fainter on the last whorl, suture well impressed; aperture rounded, broader than high, greatly indented above; lip simple, very little reflected below at its junction with the columella, very sinuous above, its terminations joined by a very thin callus. Height § inch, breadth 1 inch.

Astoria, Oregon.

In the strong rib-like sculpturing of the spire, depressed form, and sinuous lip, it resembles *sportellus.* In its greater diameter, dark greenish color, and the absence of the decussating sculpture on the last whorl, it approaches *Vancouverensis.*

All our American *Selenites* commence life with a finely granulated shell. When they have attained about two whorls, the striæ begin to appear, and increase in strength as the shell increases in size.

It is well known that all shell-bearing mollusks construct their shells in obedience to the laws of their constitutional characteristics and the environment, among which I include affinity of matter and mechanical skill, the latter a faculty possessed to a greater or less degree by all animals. Some individuals in a colony of shells display greater mechanical skill than others, or possess stronger imitative powers, and closely follow the lines and styles of their forefathers, strictly attending to the details of sculpturing, not omitting a rib or line. Other individuals of the same colony, not having this imitative faculty so strongly developed, may change or vary the form of the shell by constructing it with more convex whorls, generally resulting in a narrower or more elevated shell; or they may flatten the whorls, resulting in a broader and depressed form. Some modification of the umbilicus generally follows the change in the form of the shell. In both cases the sculpturing may be what we call characteristic of the species, or may be more or less modified by the omission of one, two, or more ribs, or the ribs may be more

irregular in shape. A few lines may also be dropped, perhaps some added, or the entire surface may be modified in obedience to the laws of the mechanical skill possessed by the individual, and the affinity of matter secreted by the animal, for the purpose of constructing the shell. An examination of a large number of *Selenites concavus*, and of our West Coast forms, convinces one that the entire group of American *Selenites* is the offspring of a single common type.

The above is Mr. Hemphill's description, from "The Nautilus," Vol. IV. p. 42, 1890.

Selenites Duranti, var. Catalinensis, Hemphill.

Plate II. Fig. 3.

I figure an authentic specimen. See Third Suppl., p. 221.

Selenites Vancouverensis, var. transfuga, Hemphill.

Shell very much depressed, planulate, broadly umbilicated, of a dirty white color; whorls 3½ or 4, flattened above, more rounded beneath, with regular strong rib-like striæ; suture well impressed, becoming deeper and channel-like as it approaches the aperture; aperture hardly oblique, slightly flattened above, with a tendency to a corresponding depression below; lip simple, roundly thickened internally, its terminations approaching, forming in some specimens a short columellar lip, joined by a heavy raised callus in very adult specimens. Height $\frac{3}{16}$ inch, greatest diameter $\frac{9}{16}$, lesser $\frac{7}{16}$ inch.

San Diego, California, to Todos Santos Bay, Lower California.

This is the small flat shell that has been distributed as a variety of *sportella*, and also as a variety of *Voyanus*. I find, however, on comparing it with the typical *Voyanus* collected by me last fall, that it is quite a different shell. The ribs are closer and finer than either *sportellus* or *Voyanus*, the umbilicus is much larger, and it is a very much more depressed shell. I consider it, however, a deserter from the Northern forms, and name it accordingly. It is a much larger and a more globose form than *simplilabris* of Ansey.

The above is Mr. Hemphill's description.

Selenites Vancouverensis, Lea.

The only differences that I can detect between this shell and *Selenites concava*, Say, are these. The umbilicus in the California shells is a little more contracted, the color is a shade darker, the striæ are a little closer, stronger, and more regular, and the body whorl is a little more flattened at the aperture. Height ⅞ inch, breadth $\frac{7}{8}$ inch.

Sonoma Co. to Santa Cruz Co., California.

The above is Mr. Hemphill's description of what he calls *S. concavus*, var. *occidentalis*.

Selenites Vancouverenis, var. tenuis, Hemphill.

Shell broadly umbilicated, depressed, nearly planulate; of a dirty greenish brown color; whorls 5, flattened above, more rounded beneath, the last expanding laterally as it approaches the aperture, and crowded with fine oblique striæ; suture well impressed; aperture rounded, slightly flattened above; peristome simple, hardly reflected below. Height $\frac{1}{8}$ inch, breadth $\frac{7}{16}$ inch.

Napa Co., California.

The small size, nearly planulate form, and thin, lean body whorl as it emerges from the aperture, will serve to distinguish this shell from the other forms of *concavus* found on the West Coast.

The above is Mr. Hemphill's description. He refers all these varieties to *concavus*, but I use the specific name *Vancouverensis* for all Pacific Region forms.

Limax Hemphilli.

Plate III. Fig. 1.

Length (contracted) 19 mm. Mantle long, 9 mm. End of mantle to end of body 9 mm. Foot wide 2 mm. Median tract of foot gray, lateral tracts brown. Median area of foot rather wider than either lateral area. Mantle free anteriorly as far as respiratory orifice. Body tapering posteriorly, not carinate. Mantle somewhat granulose, not concentrically striate. Color dark brown, obscurely marbled with gray; sides anteriorly grayish and paler.

Limax Hemphilli, W. G Binney, 3d Suppl. T. M. V., p. 205, Plate VIII. Fig. E; Plate I. Fig. 13; Plate II. Fig. 3 (1890).

A species of the Pacific Province, having been found from British Columbia to San Tomas River, Lower California, by Mr. Henry Hemphill, in whose honor it is named.

The general outward appearance of this species resembles that of *campestris*, but every specimen examined by me from numerous localities had a peculiarity in its lingual dentition which seems to me of specific value, — the presence of an inner cutting point to the lateral teeth, very much the same as is found in *agrestis*. The anatomy of this species is specifically distinct from *agrestis* in wanting the trifurcate penis sac of the latter, even did its distribution not preclude its being a form of *agrestis*. I have ventured therefore on giving it a specific name.

The penis sac is large, long, gradually tapering to the apex; the genital bladder is globular, on a short, stout duct.

I figure on the plate a variety from San Tomas River, Lower California, called *pictus* by Mr. Cockerell. Its body is pale, reticulated with gray spots; mantle with black or gray spots. Resembling *L. Berendti*, Strebel, from Guatemala.

For lingual dentition, etc., see Third Supplement.

Zonites Shepardi, Hemphill.

Shell umbilicated, very small, depressed; whorls 3 or 3½, shining, transparent, smooth, somewhat flattened; spire scarcely elevated above the body whorl; aperture oblique, oval; peristome simple, acute, its ends hardly approaching; suture well impressed; umbilicus pervious, and moderately large for so small a shell. Great diameter, 2 mm. Height, 1 mm.

Santa Catalina Island, California.

This little shell belongs to the planulate forms, and somewhat resembles a minute *Z. Whitneyi*.

I dedicate it to Miss Ida Shepard in recognition of her active services among the mollusks of Long Beach, Cal., where she resides.

The above is Mr. Hemphill's description.

Zonites Lawæ.

Shell small, umbilicated, globose, flatter below, shining, light horn-colored, marked with coarse wrinkles of growth; spire rounded; whorls 8, gradually increasing, slightly convex, the last excavated below around the umbilicus; aperture oblique, rounded; peristome simple, acute, thickened with callus within. Greater diameter 9 mm., lesser 7 mm.; height 4 mm.

Zonites placentula, part, W. G. Binney, formerly, Terr. Moll. U. S. V., p. 124, Fig. 44; Plate III. Fig. L (dentition).

Zonites Lawi, W. G. Binney, Suppl. to Vol. V. p. 142; Plate II. Fig. E (also, Ann. N. Y. Ac. Sci., Vol. I., Plate XV. Fig. E, as undetermined).

Mountains of Tennessee (Miss Law); a species of the Cumberland Subregion.

Readily distinguished from *placentula* by its larger size, higher rounded spire, greater number of whorls, and more widely excavated umbilical region.

Jaw as usual in the genus.

Lingual membrane (Vol. V. Plate III. Fig. L, as *placentula*) with 25–1–25 teeth; three laterals and one transition tooth.

Zonites Caroliniensis, Cockerell.

Plate III. Fig. 7.

Among the specimens of *Zonites sculptilis* collected in the mountains of North Carolina are many which differ from the type widely enough to be considered a distinct species. Mr. Cockerell suggests for it the name *Carolinensis*, thus describing it: —

This species differs from *sculptilis* in its fewer whorls, straighter columellar margin, less lunate aperture, fewer radiating striæ, and other points. It is figured as *sculptilis* in Manual of American Land Shells, Fig. 231.

Zonites sculptilis.

Plate III. Fig. 9.

For the sake of comparison with the preceeding species, I have given other figures here of the true *Z. sculptilis*.

Zonites Simpsoni, Pilsbry.

Plate I. Fig. 8,

I give an enlarged figure of an authentic individual of this species. For the description see Third Suppl., p. 218.

Zonites Diegoensis, Hemphill.

Plate III. Fig. 2.

Shell minute, umbilicated, thin, light horn-colored, with delicate incremental striæ, globose; whorls 3½, convex; base swollen; suture deep; umbilicus broad; aperture narrow, rounded; peristome thin, acute, its ends approximated, the inner one slightly reflected. Greater diameter 3½ mm., lesser 1½; height 1¾ mm.

Near Julian City, San Diego Co., California. On Cuyamaca Mountain, 4,500 feet elevation.

The above is Hemphill's description. My figure is drawn from an authentic specimen.

Zonites cuspidatus, Lewis.

Vol. V., Fig. in text; Suppl., Plate II. Fig. C.

Shell imperforate, small, slightly convex above, flattened below; light horn color, shining; whorls 6, gradually increasing in size, with wrinkles of growth, the last not descending at the aperture; peristome thin, acute; aperture rounded, bearing within behind the peristome a white callus, on which is one subcentral and a second basal, erect, recurved tooth-like process, separated by a rounded sinus; base often blackish, showing the white callus prominently. Greater diameter 8 mm., lesser 6; height 4 mm.

Zonites cerinoideus, var. *cuspidatus*, Lewis, Proc. Phila. Ac. Nat. Sci., 1875, p. 334.
Zonites cuspidatus, W. G. Binney, Ann. N. Y. Ac. Nat. Sci., Vol. I. p. 359, Plate XV. Fig. C; Suppl. to Terr. Moll. V., Plate II. Fig. C.

Mountains of Tennessee and North Carolina: a species of the Cumberland Subregion.

The tooth-like processes within the aperture, strongly curved towards each other, form an arched space.

Miss Law thus wrote from Philadelphia, Tenn., of this species: "Unlike *gularis*, it seems to be a rare shell, and I find it only by scraping off the surface of the ground in the vicinity of damp mossy rocks. Its habits are more like *placentula* than *gularis*. I never mistake one for a *gularis*, even before picking it up; the thickened yellow splotch near the lip, and the thinner spot behind, showing the dark animal through it, as well as its more globular form, particularly on the base, make it look very different when alive."

Zonites macilentus, Shuttl.

Plate III. Fig. 3.

The individuals of this group are very often difficult to identify, on account of the blending of their specific characters. The typical *macilentus* is distinguished by a very wide umbilicus and a single revolving lamina starting from near the basal termination of the peristome. The figure of *macilentus* in Volume V. shows a second revolving lamina and a much smaller umbilicus. I give here another figure of what appears to me to be the shell described as *macilentus*. How constant are the characters of the species can be shown only by a large suite of individuals.

Tebennophorus Hemphilli.

Plate III. Fig. 4.

I give a figure of the jaw already described by me.

Patula strigosa, Gould, var. jugalis, Hemphill.

Shell umbilicated, depressed with numerous prominent oblique striæ; spire very moderately elevated or depressed; whorls $5\frac{1}{2}$, somewhat flattened above, but more convex beneath, the last falling in front, with two dark revolving bands, one at the periphery and the other above; the body whorl subcarinated at its beginning, but more rounded as it approaches the aperture; suture well impressed; color ashy white, with occasional horn-colored stains; umbilicus large, pervious, showing the volutions; aperture oblique, ovate, but in very depressed specimens the aperture is at right angles with the axis of the shell; peristome simple, thickened, its terminations approaching and joined by a thick heavy callus, making the peristome in very adult specimens continuous. Height of the largest specimens $\frac{1}{2}$ inch, breadth 1 inch. Height of the smallest specimens $\frac{6}{16}$ inch, breadth $\frac{11}{18}$ inch.

Patula strigosa, var. *jugalis*, Hemphill, The Nautilus, 1890, p. 134, in Binney's 3d Suppl., p. 215, figure.

Banks of Salmon River, Idaho.

This is another interesting form of the very variable *strigosa*. It inhabits stone piles, and other places where it can find shelter and protection against the fatal rays of the summer's sun, close along the banks of the river. It is interesting on

account of its very depressed form and the ovate form of the aperture, the heavy callus joining or "yoking" together the extremities of the peristome.

The above is Hemphill's description.

The figure in the Third Supplement is drawn from an authentic specimen.

Patula strigosa, Gould, var. intersum, Hemphill.

Shell umbilicated, sublenticular, depressed, thin, dark horn-color, more or less stained with darker chestnut. Whorls $5\frac{1}{2}$ or 6, somewhat flattened above, more convex beneath, obtusely carinated at the periphery, and bearing numerous coarse oblique rib-like striæ, and two dark revolving bands; suture well impressed; umbilicus large, pervious; aperture oblique, subangulated; peristome simple, thickened, its terminations joined by a thick callus. Height of the largest specimen $\frac{1}{2}$ inch, breadth $\frac{3}{4}$ inch. Height of the smallest specimen $\frac{5}{16}$ inch, breadth $\frac{7}{16}$ inch.

Patula strigosa, var. *intersum*, Hemphill, The Nautilus, 1890, p. 135.

Bluffs along the banks of Little Salmon River, Idaho.

This shell inhabits stone piles at the foot of a steep bluff back some distance from the river. It seems to be quite rare, as I found but few specimens during the two or three days of my stay in its vicinity, and many of them were dead. I regard it as one of the most interesting shells found by me during the season, for it combines the depressed angulated or keeled forms of the *Haydeni* side of the series with the sculpturing of *Idahoensis*, two shells representing opposite characters in every respect. It thus becomes the companion of *Wahsatchensis*, a beautiful shell, combining the same characters, but much more developed, and connected with the large elevated forms. Var. *intersum* fills the opposite office, by uniting these characters with the small depressed forms. Taken as a whole, this series of shells, as now completed, seems to me to offer the best guide or key to the study of species that the student can have. Every known external character belonging to the genus Helix is so gradually modified and blended with opposite characters, that, if one had the moulding or making of the many and various intermediate forms, he could scarcely make the series more complete than Nature has done herself.

The above is Hemphill's description.

Patula strigosa, Gould, var. globulosa, Cockerell.

Small, globose, dark above periphery, with two bands, transverse grooved striæ rather well marked. Diameter $11\frac{1}{2}$, alt. $8\frac{1}{2}$ mm. Black Lake Creek, Summit Co. The specimen seems immature, but is remarkable as being the only form I have seen in Colorado that is nearer to *strigosa* than *Cooperi*. It is doubtless allied to var. *Gouldi*, Hemphill. (Cockerell.)

Patula strigosa, var. *globulosa*, Cockerell, The Nautilus, 1890, p. 102.

The above is Cockerell's description.

The above varieties of *Patula strigosa* are transversely ribbed. The following are smooth or striate

Patula strigosa, Gould, var. Buttoni, Hemphill.

Plate I. Figs. 2 and 10.

I figure the typical and the toothed forms. See 3d Suppl., p. 220.

Patula strigosa, Gould, var. albofasciata, Hemphill.

Plate IV. Fig. 9.

Shell globose, elevated or depressed; whorls six, convex, with a broad white band at the periphery, which shows just above the suture on two or three whorls of the spire as it passes towards the summit or apex, separating two variable chestnut-colored zones; the upper one in some specimens is often very dark, in others very light passing into horn-color, and broken into blotches, stains, or irregular lines, which pass up a few whorls of the spire and blend with the horn-colored summit; the lower zone spreads towards the umbilicus in irregular stains, often beautifully clouding the base of the shell, or is often broken into irregular revolving lines, and other varied patterns of coloring; striæ rib-like, quite coarse in some specimens, in others finer and closely set together; aperture circular, ovate, and occasionally pupæform; peristome simple, thickened, sub-reflected at its junction with the columella, and partially covering the umbilicus, the ends approached and often joined by a callus, the peristome sometimes bearing a tooth-like process; umbilicus deep, moderately large, narrower in elevated and broader in depressed specimens; suture well defined. Greater diameter of the largest specimen 17 mm., height, 12 mm.; greater diameter of the smallest 12 mm., height 7 mm.; with all the intermediate sizes.

Box Elder Co., Utah.

Among leaves, brush, and grass, on limestone rock. Altitude, about 4,600 feet above the sea.

This variety of *strigosa* is so very variable in all its characters I find it quite difficult to draw a description that will cover all the individuals which I include in it. I have given the measurements of the largest and smallest specimens, but there are all the intermediates between those figures.

The above is Mr. Hemphill's description. An authentic individual is figured on the plate.

Patula strigosa, Gould, var. subcarinata, Hemphill.

Among the shells recently collected by Mr. Hemphill at Old Mission, Cœur d'Alene, Idaho, was a marked variety of this species, for which Mr. Hemphill suggests the name *subcarinata*. The specimens vary greatly in elevation of the spire, and in the number and disposition of the revolving bands, often quite wanting, as in the specimen figured in the Third Supplement. All have a very heavy shell, the body whorl of which has an obsolete carina which is well marked at the aperture, modifying the peristome very decidedly. See the figure.

In examining the genitalia I find the base of the duct of the genital bladder greatly swollen along a fifth of the total length of the duct.

Mr. Hemphill (The Nautilus, 1890, p. 133) thus describes it : —

The shell in general form resembles a large, coarse elevated or depressed *Cooperi*. It has six whorls, well rounded above and beneath, and subcarinated at the periphery. The body whorl has two revolving dark bands, one above and the other below the periphery; sometimes the upper band spreads over the shell to the suture, forming a dark chestnut zone that fades out as it passes toward the apex. The peristome is simple, thickened, its terminations joined by a callus; aperture obliquely subangulate; the suture is well impressed. Height of the largest specimen 1 inch, breadth 1½ inches; height of the smallest specimen ¾ inch, breadth 1 inch.

Rathdrum, Idaho.

An authentic specimen is figured in the Third Supplement.

Patula strigosa, Gould, var. bicolor, Hemphill.

Plate IV. Fig. 7.

This shell is a colored variety of the last. It may be characterized as being of a general dark horn-color mingled with dirty white; there are occasional zones of dark horn-color above and fine dark lines beneath, but no defined bands. In some of the specimens the light color prevails, in others the horn-color spreads over the shell in irregular patches. Height ⅞ inch, breadth 1⅛ inches.

Rathdrum, Idaho. (Hemphill.)

Patula strigosa, var. *bicolor*, Hemphill, The Nautilus, 1890, p. 133.

An authentic specimen is figured.

Patula strigosa, Gould, var. lactea, Hemphill.

Plate IV. Fig. 8.

This is a beautiful clear milk-white shell, with 5½ whorls, subcarinated at the periphery. In the elevated forms the aperture is nearly circular, as broad as high; but in the depressed forms the aperture is broader than high, obliquely subangulate. The lip is simple, thickened, its terminations joined by a heavy callus, — the thickening of the lip and callus is a shade darker than the body of the shell. Height of the largest specimen 1 inch, breadth 1⅛ inches.

Rathdrum, Idaho.

The above varieties represent a colony of the largest specimens of the *strigosa* group that I have collected. They are an important and very interesting addition to the series, and serve to confirm my previous views on the relationship of what I call the *strigosa* group. This colony inhabits open places in the dense pine forests of the mountains, overgrown with deciduous bushes. They hibernate among

leaves, brush, and roots of trees, and in protected and secure places, generally on the north slopes of the mountains. (Hemphill.)

Patula strigosa, var. *lactea*, HEMPHILL, The Nautilus, 1890, p. 134.

An authentic specimen is figured.

Patula strigosa, var. Utahensis, HEMPHILL.

For locality, see 2d Supplment, p. 30. This is a rough, coarse, carinated variety, figured in Terr. Moll. V., p. 158, Fig. 66. The peristome is sometimes continuous by a heavy raised callus connecting its terminations. It is sometimes smaller and more elevated. (2d Suppl., p. 33.)

Patula strigosa, GOULD, var. depressa, COCKERELL.

Shell flattish, maximum diameter 21⅓, altitude 12½ mm. Specimens of this variety were sent to me by Miss A. Eastwood, who found them in a cañon near Durango, Colorado. The same variety is figured by Binney, Man. Amer. Land Shells (1885), p. 166, Fig. 153. (Cockerell.)

Patula strigosa, var. *depressa*, COCKERELL, The Nautilus, 1890, p. 102.

Patula strigosa, var. albida, HEMPHILL.

Shell broadly umbilicated, greatly depressed, white, tinged with horn-color; surface covered with fine oblique striæ and fine microscopic revolving lines; whorls 6, convex, the last falling in front; spire very little elevated, apex obtuse, aperture oblique, nearly round; peristome simple, thickened, subreflected at the columella, its terminations approaching, joined by a thin callus. Height ½ inch, greatest diameter 1 inch, lesser ⅜ inch.

Near Logan, Utah.

Patula strigosa, var. *albida*, HEMPHILL, The Nautilus, IV. p. 17, June, 1890.

The above is Hemphill's description.

Patula strigosa, var. parma, HEMPHILL.

Shell broadly umbilicated, greatly depressed, of a dark dirty horn-color, surface somewhat rough, covered with coarse irregular striæ, and microscopic revolving lines; whorls 5½ or 6, subcarinated throughout, somewhat flattened above, rounded beneath, and striped with two chestnut-colored bands, one above and the other just at the periphery; spire very little elevated, umbilicus moderately large and deep; aperture ovately round, oblique; peristome simple, subreflected, its terminations approaching and joined by a thin callus. Height ½ inch, breadth 1 inch.

Near Spokane Falls, Washington.

Patula strigosa, var. *parma*, HEMPHILL, The Nautilus, IV. p. 17, June, 1890.

The above is Hemphill's description.

Patula strigosa, var. rugosa, Hemphill.

Shell umbilicated, elevated or globosely depressed, of a dull brown ash-color; surface rough, covered with coarse irregular oblique striæ, and microscopic revolving lines; whorls 5, convex, with or without one or two narrow faint revolving bands. In most of the specimens the bands are obsolete; spire elevated, obtusely conical; suture well impressed; umbilicus large, deep; aperture nearly round; peristome simple, thickened, its terminations approaching and joined by a thin callus. Height of the largest specimen $\frac{3}{4}$ inch, greatest diameter 1 inch. Height of the smallest specimen $\frac{1}{2}$ inch, greatest diameter $\frac{3}{4}$ inch.

New Brigham City, Utah.

A large rough robust form, with very convex whorls. Some of the specimens so closely resemble *solitaria*, Say, that one not well acquainted with both forms would be easily deceived, and refer it to that species. In its adolescent state the lip is very thin or easily broken, and on the surface of the adult shells these fractures give it a rough and uneven appearance.

Patula strigosa, var. *rugosa*, Hemphill, The Nautilus, 1890, Vol. IV. p. 16.

The above is Hemphill's description.

Patula strigosa, var. carnea, Hemphill.

Shell umbilicated, greatly depressed, dark horn-color, rather solid, shining, surface somewhat uneven and covered with irregular oblique striæ; whorls $5\frac{1}{2}$, convex, the last faintly subcarinated in the depressed specimens, falling in front, sometimes faintly banded, but most of the specimens are plain and without bands; spire subconical, apex obtuse; suture well impressed, umbilicus large; aperture circular; peristome simple, thickened, its terminations well approached and joined by a callus. Height $\frac{5}{8}$ inch, greater diameter $\frac{7}{8}$, lesser $\frac{3}{4}$ inch.

Near Salt Lake, Utah.

Patula strigosa, var. *carnea*, Hemphill, The Nautilus, Vol. IV. p. 15, June, 1890.

The above is Hemphill's description.

Patula strigosa, var. fragilis, Hemphill.

Shell umbilicated, elevated or globosely depressed, translucent, thin, fragile, somewhat shining, of a dark horn-color, surface covered by fine oblique striæ; whorls 5, convex, the last descending in front and striped by two dark chestnut bands, one above and the other below the periphery; suture well impressed; aperture oblique; peristome simple, thickened; umbilicus moderate, deep, partially covered by the reflected peristome at the columella. Height of the largest specimen $\frac{9}{16}$ inch, greatest diameter $\frac{7}{8}$ inch, lesser $\frac{3}{4}$ inch.

Near Franklin, Idaho, among red sandstone.

A very thin and almost transparent variety of the very variable *strigosa*. By its

peculiar shade, it is very evident that the animal has drawn largely from the red sandstone for the material to build its shell.

Patula strigosa, var. *fragilis*, HEMPHILL, The Nautilus, Vol. IV. p. 17, June, 1890.

The above is Hemphill's description.

Patula strigosa, var. picta, HEMPHILL.

Shell umbilicated, elevated or globosely depressed, of a dirty white color, stained more or less with chestnut; surface somewhat rough and uneven, covered with moderately coarse oblique striæ, and fine revolving lines; whorls 6, convex, subcarinated, with a broad white band at the periphery, and a dark zone of chestnut on the upper side, extending from the peripheral band to the suture, fading out as it traverses the whorls of the spire; beneath, on the base of the shell, it is striped with numerous bands that sometimes extend into the umbilicus, and also into the aperture; spire elevated; apex obtuse; suture well impressed; umbilicus moderately large and deep, broader in the depressed than in the elevated forms; aperture nearly circular; lip simple, subreflected, its terminations approaching and joined by a thin callus. Height $\frac{7}{8}$ inch, greatest diameter $1\frac{1}{8}$ inches, lesser 1 inch.

Rathdrum, Idaho.

Patula strigosa, var. *picta*, HEMPHILL, The Nautilus, Vol. IV. p. 16, June, 1890.

The above is Hemphill's description.

Patula strigosa, var. hybrida, HEMPHILL.

Shell umbilicated, depressed, white, spire horn-color, surface of the shell covered with fine oblique striæ, and widely separated revolving raised lines; whorls 5, flattened above, rounded beneath, the last falling in front, and striped with two faint chestnut bands; suture well impressed; umbilicus large, showing nearly all the volutions; aperture nearly circular; peristome simple, thickened, its terminations approaching and joined by a thin callus. Height $\frac{3}{8}$ inch, diameter $\frac{5}{8}$ inch, lesser $\frac{3}{8}$ inch.

Near Logan, Utah.

This is an interesting shell, as it is the beginning of the forms of *strigosa* that finally develop the revolving lines into prominent ribs, as seen on the surface of var. *Haydeni*, Gabb.

Patula strigosa, var. *hybrida*, HEMPHILL, The Nautilus, Vol. IV. p. 17, June, 1890.

The above is Hemphill's description.

Mr. Cockerell (The Nautilus, 1890, p. 102) mentions by name only the following Colorado forms:—

P. strigosa Cooperi, form *trifasciata*, Ckll. Mesa Co.

P. strigosa Cooperi, form *confluens*, Ckll. West Mountain Valley, Custer Co.; Garfield Co.; Mesa Co.

P. strigosa Cooperi, form *elevata*, Ckll. Delta Co.

P. strigosa Cooperi, form *major*, nov. Shell with diam. 25 mm. Near head of North Mam Creek, Mesa Co., Sept. 14, 1887.

P. strigosa Cooperi, var. *minor*, Ckll. Near Egeria, Routt Co., abundant. It is quite a distinct local race.

Pristiloma, Ancey.

Animal as in Patula.

Shell small, imperforate, horn-color, shining, many whorled; spire depressed conic; aperture sometimes armed with radiating, rather crowded, palatal lamellæ.

Northern and Arctic North America.

Types: *Zonites Stearnsi* and *Lansingi*, Bland.

Formerly *Pristina*, Ancey, and *Anceyia*, Pilsbry, preoc.

Jaw low, wide, slightly arcuate, ends little attenuated, blunt, with numerous crowded broad ribs, denticulating either margin.

Lingual membrane with tricuspid centrals, bicuspid laterals, aculeate marginals, as in *Zonites*.

Separated from *Microphysa* by the ribbed jaw combined with the lingual membrane of *Zonites*: a very unusual occurrence.

Pristina Lansingi, Bland.

Plate III. Fig. 6.

I give a better figure of this species.

Pristiloma Stearnsi, Bland.

Vol. V., figures in text. Suppl., Plate I. Figs. N (dentition) and O (jaw).

Shell minute, imperforate, globose conic, striate, shining, horn-colored; suture impressed; whorls 7, regularly increasing, the last not descending, very globose, swollen below, excavated closely around the imperforate umbilical region; aperture rounded; peristome simple, acute. Greater diameter 4 mm., lesser $3\frac{1}{2}$; height $2\frac{1}{2}$ mm.

Zonites Stearnsi, Bland, Ann. N. Y. Lyc., XI. 74, Figs. 1, 2 (1875).

Microphysa Stearnsi, W. G. Binney, Terr. Moll. V., figs. in text; Suppl., Plate II. Figs. N (dentition) and O (jaw).

Astoria, Portland, Oregon; Olympia, Washington; Alaska. A species of the Oregonian region.

It is larger, more elevated, and more distinctly striated than *Lansingi*, with wider, more rounded, unarmed aperture.

The jaw is of the same type as described under *P. Lansingi*, with over 19 ribs. (Suppl., Plate II. Fig. O.)

The peculiar lingual membrane also is the same as in that species, with four laterals on each side of the central tooth. (Suppl., Plate I. Fig. N.)

Punctum, Morse.

Animal as in *Patula*.

Shell minute, umbilicated, thin, horn-colored, depressed globose; whorls 4, the last not descending; spire slightly elevated; aperture rounded; peristome thin, acute.

Europe and North America.

Jaw slightly arcuate, ends blunt, not acuminated, composed of numerous subequal, overlapping distinct plates.

Lingual membrane as usual in the *Helicidæ;* bases of attachment subquadrate, reflection small, tricuspid in the centrals, bicuspid in the laterals, marginals irregularly denticulated.

Distinguished by the peculiar free plates of the jaw.

There are two species of *Punctum*, *conspectum* and *pygmæum*.

Helicodiscus fimbriatus, Wetherby, var. salmonaceus, Hemphill.

Plate III. Fig. 8.

I give a figure of this variety from an authentic specimen. See 3d Suppl., p. 189.

Anadenus, Heynemann.

Animal limaciform, subcylindrical, tapering behind; tentacles simple; mantle anterior, concealing an internal shell-plate; no longitudinal furrows above the margin of the foot, and no caudal mucus pore; a distinct locomotive disk; external respiratory and anal orifices on the right posterior margin of the mantle; orifice of combined genital system behind and below the light eye-peduncle. (See Plate I. Fig. 1.)

Internal shell-plate small, oval, flat, with posterior nucleus and concentric striæ. (See Plate.)

Jaw with numerous ribs. See Plate III. Fig. 5.

Lingual membrane with tricuspid centrals, bicuspid laterals, and quadrated marginals. (See same.)

Differs from *Prophysaon* by its posterior respiratory orifice, by the position of the genital orifice, and by its locomotive disk.

Himalaya Mountains; recently found in San Diego County, California, by Mr. Hemphill.

It will be remembered that Fischer considers *Prophysaon* a subgenus of *Anadenus*.

The geographical distribution of *Anadenus* would seem to preclude its being found in California, but to that genus only can I refer the species whose description here follows.

Anadenus Cockerelli, Hemphill.

Plate I. Fig. 1; Plate III. Fig. 5.

Length (contracted) 13½ mm.; mantle, length 4½, breadth 2¾ mm. End of mantle to end of body, 8 mm. Foot, breadth 2 mm. Foot with the locomotive disk, being distinctly differentiated into median and lateral tracts. Respiratory orifice slightly posterior on right side of mantle. Genital orifice below right tentacle. No caudal mucus pore. Locomotive disk about half as wide as either lateral area. Sides of foot wrinkled, but not differentiated from lateral areas, nor specially marked, the wrinkles being a continuation of the transverse grooves of the lateral areas. Mantle tuberculate-rugose, oval in outline, bluntly rounded at either end; not grooved as in *Amalia*. Mantle free in front as far as respiratory orifice. Back rather bluntly keeled its whole length; rugæ rather flattened and obscure, consisting of grooves enclosing mostly hexagonal lozenge-shaped spaces, which are themselves rugose. Color uniform brown-black, without markings, except some dark marbling on the lighter sides. The portion beneath and in front of the mantle is pale, and the head and neck have a gray tinge. Foot brown. Shell internal, thinnish, white, oval in outline. Stomach large, swollen, broad. Liver pale ochrey.

Anadenus Cockerelli, Hemphill, The Nautilus, Vol. IV. No. 1, May, 1890, p. 2.
Anadenulus, Cockerell, Ann. Mag. Nat. Hist., Oct., 1890, p. 279.

Cuyamaca Mountains of San Diego Co., California. Mr. Henry Hemphill.

Jaw low, wide, slightly arcuate, ends blunt, anterior surface with about twenty wide, flat ribs, squarely denticulating either margin. (Plate III. Fig. 5.)

Lingual membrane short and narrow. Teeth 20–1–20, of which eight only on either side are laterals. Centrals tricuspid, laterals bicuspid, marginals quadrate, bluntly bicuspid. (Same Plate.)

Prophysaon Hemphilli.

From Portland, Oregon, Mr. Hemphill brought seventy-seven individuals of a slug which may prove a variety of *P. Hemphilli*. They have the tawny color of *flavum*. The internal shell is so delicate, it is impossible to remove it without breaking it. The penis sac is as in *P. Hemphilli*. The mantle is sometimes smooth, sometimes tuberculate; its fuscous lateral bands are sometimes united by a transverse posterior band. Some of the individuals had the tail constricted preparatory to excision. (See below, under *Phenacarion*.)

Prophysaon Andersoni, J. G. Cooper.

3d Suppl., Plate III. Fig. 1? Plate VII. Fig. C; Plate I. Fig. 3 (dentition); Plate IX. Figs. I, J (enlarged surface).

Shield strongly granular-rugose, the respiratory orifice nearly median on its right margin; tail acute, with small gland; reddish gray, the body somewhat clouded with black, the shield paler, clouded, or more usually with a dark band on each side above the respiratory orifice, converging in an elliptic form; a pale dorsal streak; head uniform pale brown, tentacles darker; foot and often the mantle tinged with olive. Length 2.5 inches (Cooper).

Arion Andersoni, J. G. Cooper, Proc. Phila. Ac. Nat. Sci., Plate III. Fig. F.
Prophysaon Andersoni, J. G. Cooper, Pr. Amer. Phil. Soc., 1879, p. 288.
Prophysaon Andersoni, W. G. Binney, Terr. Moll. V., 3d Suppl., Plate III. Fig. 1? Pl. VII. Fig. C; Plate I. Fig. 3 (dentition); Plate IX. Figs. I, J (surface).

A species of the Pacific Province, Straits of De Fuca to Oakland, California.

The characteristic of this species is the light dorsal band, which is not present in *P. Hemphilli*. It has the broad vagina, stout, short, cylindrical penis sac, and genital bladder of *P. Hemphilli*, as well as the foliated reticulations.

In the many living and alcoholic specimens which I have examined, I have failed to detect any appearance of a caudal mucus pore, which Dr. Cooper is confident of having observed, excepting in eight individuals out of thirty collected by Mr. Hemphill on San Juan Island.

Many individuals examined by me are excided as described under *Phenacarion foliolatus*.

Figure 1 of Plate III. of 3d Suppl. was drawn from a specimen received from Dr. Cooper. It represents the true *Andersoni*, distinguished by a light dorsal band, and by genitalia such as I have described for *P. Hemphilli*. The same form, also received from Dr. Cooper, is drawn by Mr. Cockerell on Plate VII. Fig. C. Mr. Cockerell has shown me that I have confounded with it another species, which he proposes to call *P. fasciatum*. See next species.

Specimens collected by Mr. Hemphill at Old Mission, Cœur d'Alene, Idaho, appear to agree with specimens of this species received from Dr. Cooper. The jaw is low, wide, slightly arcuate, with over 12 broad, stout ribs, denticulating either margin. The lingual membrane is given in Plate II. Fig. 2, of 3d Suppl. The central and lateral teeth are slender and graceful. The latter have, apparently, a second inner cutting point, as is found in *Limax agrestis*. I have so figured it, hoping to draw attention to it, and thus settle the question of its being there. On Plate IX. I have given enlarged views of the surface, drawn by Mr. Arthur F. Gray. (See Explanation of Plate IX. Figs. I and J of 3d Suppl.)

Prophysaon fasciatum, Cockerell.

Length (in alcohol) 19 mm. Mantle black, with indistinct pale subdorsal bands, — an effect due to the excessive development of the three dark bands of the mantle. Body with a blackish dorsal band, commencing broadly behind mantle and tapering to tail, and blackish subdorsal bands. No pale dorsal line. Reticulations on body squarer, smaller, more regular, and more subdivided than in *P. Andersoni*, Cooper. Penis sac tapering, slender. Testicle large. Jaw ribbed. (Cockerell.)

Prophysaon fasciatum, Cockerell, The Nautilus, 1890.
Prophysaon fasciatum, W. G. Binney, 3d Suppl. to Terr. Moll. V., p. 209, Plate VII. Fig. A.

Cœur d'Alene Mountains, Idaho; a species of the Central Region.

This species is described by Mr. Cockerell as distinct from *Andersoni*, with which I have formerly confounded it. (2d Suppl. to Vol. V., p. 42.) It has a dark band on each side of the body, running from the mouth to the foot, and a central dorsal dark band. To this must be referred the descriptions of animal, dentition, jaw, and genitalia formerly published by me as of *Andersoni*.

I am indebted to Mr. Theo. D. A. Cockerell for a figure and description of this species. The former is given on Plate VII. Fig. A, while the latter is given here in the words of Mr. Cockerell, whose name must consequently be associated with it as authority.

The animal extends itself into a long, cylindrical worm-like body with obtuse ends; the mantle is covered with minute tubercles.

Jaw low, arcuate, ends blunt; with numerous (over 15) irregularly developed broad, stout ribs, denticulating either margin.

The lingual membrane has 30–1–30 teeth, with about 12 perfect laterals. Centrals tricuspid; laterals bicuspid; marginals with one long, stout, oblique inner cutting point, and one outer short, blunt, sometimes bifid cutting point. Resembling that of *P. Hemphilli*. Another membrane has 50–1–50 teeth.

Mr. Cockerell describes the penis sac as tapering; in specimens examined by me it is cylindrical, as in *Hemphilli*.

The internal shell is thick, easily extracted without breaking.

Phenacarion, Cockerell.[1]

Animal limaciform, cylindrical, blunt before, tapering behind; tentacles simple; mantle large, anterior, pointed behind, concealing a delicate, thin, subrudimentary calcareous shell-plate, easily fractured; no longitudinal furrows along the margin of the foot; a caudal mucus pore; no distinct locomotive disk; external respiratory and anal orifices on the right anterior margin

[1] *Phenax* = an impostor, and *Arion*. Cockerell, The Nautilus, Vol. III. p. 126, March, 1890.

of the mantle; orifices of the combined generative organs behind and below the right eye-peduncle. (See 3d Suppl., Plate VIII. Fig. A.)

Jaw arcuate, with numerous ribs. (Plate IX. Fig. B of same.)

Lingual membrane with tricuspid centrals, bicuspid laterals, and quadrate denticulated marginals. (Plate IX. Fig. C of same.)

Northwestern parts of North America, in the Oregon Region.

Allied to *Prophysaon*, but distinguished by its more anterior respiratory orifice, its rudimentary shell-plate, and decided caudal pore.

Phenacarion foliolatus, Gould.

Color a reddish fawn, coarsely and obliquely reticulated with slate-colored lines, forming areolæ, which are indented at the sides, when viewed by a magnifier, so as to resemble leaflets; the mantle is concentrically mottled with slate-color, and the projecting border of the foot is also obliquely lineated. The body is rather depressed, nearly uniform throughout, and somewhat truncated at the tip, exhibiting a conspicuous pit, which was probably occupied by a mucus gland. The mantle is very long, smooth, and has the respiratory orifice very small, situated a little in front of the middle. The eye-peduncles are small and short. Length 85 mm.

Arion foliolatus, Gould, Moll. U. S. Exp., page 2, Fig. 2, *a*, *b* (1852); Binney, Terr. Moll., II. 30, Plate LXVI. Fig. 2 (1851); W. G. Binney, Terr. Moll., IV. 6; copied also by Tryon and W. G. Binney, L. & Fr. W. Sh., I. 377.

Phenacarion foliolatus, Cockerell, The Nautilus, 1890, III. 126; W. G. Binney, 3d Suppl. to Terr. Moll. V., p. 206, Plate VIII. Fig. A; Fig. B (shell-plate); Plate IX. Fig. B (jaw); Fig. C (dentition); Fig. D (genitalia).

Discovery Harbor, Puget Sound (Pickering); Olympia and Seattle, Washington (Hemphill).

Dr. Gould adds to the above description these words (Vol. II. p. 31): "That this animal belongs to the genus *Arion* there can be little doubt, from the peculiar structure of the tail, as represented in Mr. Drayton's figure, and from the anterior position of the respiratory orifice. It is a well marked species, characterized especially by the leaf-like areolæ by which the surface is marked."

It is with the greatest pleasure that I announce the rediscovery by Mr. Henry Hemphill of this species, which has hitherto escaped all search by recent collectors. It has till now been known to us only by the description and figure of the specimen collected by the Wilkes Exploring Expedition, almost fifty years ago, and given in Vols. II. and III. of Terrestrial Mollusks. A single individual was found in December, 1889, at Olympia, Washington, and sent to me living by Mr. Hemphill. It can thus be described. (See Fig. A of Plate VIII. of 3d Suppl.)

Animal in motion fully extended over 100 millimeters. Color a reddish

fawn, darkest on the upper surface of the body, mantle, top of head, and eye-peduncles, gradually shaded off to a dirty white on the edge of the animal, side of foot, back of neck, and lower edge of mantle, and with a similar light line down the centre of back; foot dirty white, without any distinct locomotive disk; edge of foot with numerous perpendicular fuscous lines, alternating broad and narrow; mantle minutely tuberculated, showing the form of the internal aggregated particles of lime, the substitute of a shell-plate, reddish fawn-color, with a central longitudinal interrupted darker band and a circular marginal similar band, broken in front, where it is replaced by small, irregularly disposed dots of same color; these dots occur also in the submarginal band of light color. Body reticulated with darker colored lines, running almost longitudinally, scarcely obliquely, toward the end of the tail, and connected by obliquely transverse lines of similar color, the areas included in the meshes of this network covered with crowded tubercles, as in *Prophysaon Andersoni*, shown in Plate IX. Figs I, J. Tail cut off by the animal. (See below.) Excepting its being of a deeper red, it agrees perfectly with Dr. Gould's description.

Mr. Hemphill writes of it: "I have to record a peculiar habit that is quite remarkable for this class of animals. When I found the specimen, I noticed a constriction about one third of the distance between the end of the tail and the mantle. I placed the specimen in a box with wet moss and leaves, where it remained for twenty-four hours. When I opened the box to examine the specimen, I found I had two specimens instead of one. Upon examination of both, I found my large slug had cut off his own tail at the place where I noticed the constriction, and I was further surprised to find the severed tail piece possessed as much vitality as the other part of the animal. The ends of both parts at the point of separation were drawn in as if they were undergoing a healing process. On account of the vitality of the tail piece, I felt greatly interested to know if a head would be produced from it, and that thus it would become a separate and distinct individual." The animal on reaching me still plainly showed the point of separation from its tail (see Fig. A). The tail piece was in an advanced stage of decomposition. I have noticed the constriction towards the tail in many individuals. The edges of the cut were drawn in like the fingers of a glove, after the excision.

The tail of the *foliolatus* having been cut off, I was unable to verify the presence of a caudal pore from this individual. It was plainly visible in another specimen from Seattle.

In the large Olympia individual, the irregularly disposed particles of lime in the mantle, of unequal size, seemed attached to a transparent membranous plate. With care I removed this entire, and figure it. It is suboctagonal in shape (Plate VIII. Fig. B). Under the microscope it appears that the particles of lime do not cover the whole plate; at many points they are widely separated. This aggregation of separate particles is the distinctive character of the subgenus *Prolepis*, to which *foliolatus* would belong if retained in *Arion*.

The genitalia of the large individual from Olympia is figured on Plate IX. Fig. D. The ovary is tongue-shaped, white, very long and narrow; the oviduct is greatly convoluted; the testicle is black in several groups of cœca; the vagina is very broad, square at the top with the terminus of the oviduct, and the duct of the genital bladder entering it side by side; the genital bladder is small, oval, on a short narrow duct; the penis sac is of a shining white color, apparently without retractor muscle; it is short, very stout, blunt at the upper end where the extremely long vas deferens enters, and gradually narrowing to the lower end. There are no accessory organs. The external orifice of the generative organs is behind the right tentacle. (See 3d Suppl., Plate IX. Fig. D.)

The jaw is very low, wide, slightly arcuate, with ends attenuated and both surfaces closely covered with stout, broad separated ribs, whose ends squarely denticulate either margin. There are about 20 of these ribs. (See Plate IX. Fig. B.)

The lingual membrane is long and narrow, composed of numerous longitudinal rows of about 50–1–50 teeth, of which about 16 on each side (Plate IX. Fig. C) may be called laterals. Centrals tricuspid, laterals bicuspid, marginals with one long inner stout cutting point, and one outer short side cutting point. The figure shows a central tooth with its adjacent first lateral, and four extreme marginals.

Phenacarion Hemphilli.

This form is figured on Plate VIII. Fig. C of 3d Suppl. When extended fully, it is 70 mm. long. It is more slender and more pointed at the tail than *foliolatus*. The body is a bright yellow, with bluish black reticulations. The edge of the foot and the foot itself are almost black; shield irregularly mottled with fuscous; the body also is irregularly mottled with fuscous, and has one broad fuscous band down the centre of the back, spreading as it joins the mantle, with a narrower band on each side of the body. The other characters, external and internal, are given below. It loses its color on being placed in spirits, becoming a uniform dull slate-color. Mantle lengthened oval. Shell-plate represented by a group of calcareous grains concealed in the mantle; it is impossible to remove it as one shell-plate. A decided caudal pore.

Phenacarion foliolatus, var. *Hemphilli*, W. G. Binney, 3d Suppl. to Terr. Moll. V., p. 208; Plate VIII. Fig. C; Plate X. Fig. H (genitalia).

Gray's Harbor and Chehalis, Washington, and Portland, Oregon (Hemphill); a species of the Oregon Region.

On the only living one of the lot from Gray's Harbor, the pore was distinctly visible, and is figured on Plate VIII. Fig. C. Usually it seemed more "a conspicuous pit" than a longitudinal slit, as in *Zonites*. At one time I distinctly saw a bubble of mucus exuding from it. It opened and shut, and is

still plainly visible on the same individual, which I have preserved in alcohol and added to the Binney Collection of American Land Shells in the National Museum at Washington.

Jaw low, wide, arcuate, ends attenuated, anterior surface with 16 ribs, denticulating either margin.

Lingual membrane as in *foliolatus;* teeth 50–1–50, with 19 laterals on each side.

Genitalia (3d Suppl., Plate X. Fig. H); the form from Gray's Harbor has its generative system very much the same as described for *foliolatus* above. The ovary is much shorter and tipped with brown, and is less tongue-shaped. The penis sac tapers to its upper end. The vagina is not squarely truncated above. The system much more nearly resembles that of *Prophysaon Andersoni* (see Terr. Moll., V.) than that of the Olympia *foliolatus*.

Binneya notabilis, J. G. Cooper.

Plate I. Fig. 9.

A new figure is here given, drawn by Mr. Cockerell.

Triodopsis Mullani, Bland, var. Blandi, Hemphill.

Plate II. Fig. 6.

Shell with the umbilicus partially closed, orbicularly depressed; dark horn-color, obliquely striated; spire short, very slightly elevated, nearly planiform; aperture semilunar, at a right angle with axis of the shell, with a very short nipple-like parietal tooth; peristome thickened, white, plain, without teeth and roundly reflected. Height $\frac{1}{4}$ inch, breadth $\frac{1}{2}$ inch.

Post Falls, and banks of Salmon River, Idaho.

Helix Mullani in form and size resembles very much the common *tridentata* of the Eastern States. Among the various forms it assumes, none are more marked than the little depressed shell before me. It can be very readily separated from the typical *Helix Mullani*, or its other varieties, by its very depressed form, small size, and the absence of the teeth-like processes on the inner margin of the peristome.

I cannot detect any microscopical revolving lines, or tubercles bearing hairs, mentioned by Bland in his description of *H. Mullani*.

The above desciption is by Mr. Hemphill, who furnished me with the specimen figured.

Polygyra septemvolva, var. Floridana, Hemphill.

Shell deeply umbilicated, elevated, globose conic, light horn-color, with numerous fine ribs above, but smooth beneath; whorls $5\frac{1}{2}$ or 6, the last subangular at the periphery; suture well impressed; spire greatly elevated with an obtuse apex;

aperture lunate, well rounded, and nearly circular; peristome reflected, rounded in front, the margins joined by a triangular tooth on the parietal wall. Greater diameter 6 mm., altitude 5 mm.

Oyster Bay, Florida.

This is a small, very elevated form of the *P. cereolus* group.

The above is Mr. Hemphill's description.

Mesodon ptychophorus, A. D. Brown, var. castaneus, Hemphill.

Shell umbilicated, globosely depressed, of a dark chestnut color; surface covered with coarse, irregular, widely separated lines of growth, and crowded, microscopical revolving lines; whorls 5½, convex, the last slightly descending in front, spire elevated; suture well impressed, aperture subcircular, lip white, reflected and partially covering the umbilicus, its terminations approaching; umbilicus small and deep. Height ⅝ inch, diameter 1 inch.

Old Mission and Rathdrum, Idaho.

I regard *H. ptychophorus* as the progenitor of what I call the *Townsendiana* group of West Coast land shells, and this colored variety seems to still further indicate its relationship to *Townsendiana*, for the spire whorls of nearly all the specimens of *Townsendiana* that I have collected are chestnut-colored. *Townsendiana* does not begin to put on its wrinkles until it has made about four revolutions of the shell. The wrinkles are probably due to its environment.

The above is Hemphill's description, from The Nautilus, Vol. IV. p. 41, 1890.

Aglaja fidelis, var. flavus, Hemphill.

Shell umbilicated, elevated, very faintly subcarinated, of a uniform light yellow color throughout, without bands or other stains of coloring; whorls 6½, convex, with coarse oblique striæ, and microscopic irregular revolving lines; peristome reflected below, simple above; aperture roundly ovate; umbilicus moderate, and partially covered by the reflected peristome; suture distinct. Greater diameter 34 mm., altitude 23 mm.

Chehalis and San Juan Islands, Washington; Port Orford, Oregon.

This is a rare and beautiful variety of this well known West Coast land snail.

The above is Mr. Hemphill's description.

Aglaja fidelis, var. subcarinata, Hemphill.

Shell orbicularly depressed; umbilicated; of a deep dark chestnut-color without bands; whorls 6½, convex or somewhat flattened, the last subcarinated at the periphery; striæ coarse, oblique, crossed by numerous well defined wavy revolving lines; peristome simple, thickened above, reflected below, and nearly covering the umbilicus; umbilicus moderate; aperture roundly ovate; suture well impressed. Greater diameter 37 mm., altitude 20 mm.

Humboldt Co., California.

This is a very dark, intermediate form of *fidelis*, which in its southern march under changed conditions assumes a more carinated form, and is known to conchologists as *infumata*, Gould.

The above is Mr. Hemphill's description.

Arionta Coloradoensis, Stearns.

Shell orbicular, moderately depressed, whorls slightly elevated, apex obtuse, number of whorls four to four and a half, rounded. Umbilicus narrow, showing the penultimate whorl, though partially covered by the reflection of the lip at the point of junction with the base of the shell. Aperture obliquely ovate, nearly circular, and almost as broad as high. Lip slightly thickened and reflected, or simple, varying in this respect; more reflected and aperture more effuse at the columella.

Parietal wall in the heavier examples calloused, the callus connecting with the inner edges of the outer lip above and below. Shell rather fragile, thin, translucent; surface smooth and shiny, and sculptured with fine incremental lines. Color pale horn to white, and otherwise marked by a single narrow revolving reddish brown band just above the periphery, which in some specimens is obscure or absent. In some individuals certain faint scars upon the upper whorls imply an occasionally hirsute character.

	mm.
Maximum diameter of largest	15.25
Minimum diameter of largest	13.25
Altitude of largest	10.25
Maximum diameter of smallest adult	13.75
Minimum diameter of smallest adult	12.00
Altitude of smallest adult	8.75

Grand Cañon of the Colorado, opposite the Kaibab plateau, at an elevation of 3,500 feet. (Mus. No. 104,100.)

The above, while exhibiting a facies or aspect of its own, its nevertheless suggestive of *H. Remondi*, Gabb, Mazatlan, in the Mexican State of Sinaloa, and also from the high mesas or table lands in the neighborhood of Mulege, Lower California. *H. Carpenteri*, Newcomb, which is a synonym of *H. Remondi*, is credited by the author to "Tulare Valley," and has been found in other localities in Cali-

fornia. A glance at the map will show how widely separated geographically *H. Coloradoensis* is from its nearest allies, and this discovery of Dr. Merriam's extends the distribution of the West Coast type of *Helices* farther to the eastward than heretofore, and adds an area of great extent to that previously known.

The above description and figure were published by Stearns in Proc. U. S. Nat. Mus., Vol. XIII. p. 206, Plate XV. Fig. 6, 7, 8, 1890, all copied above.

I have examined the jaw and lingual dentition to find them similar to those of the other species of *Arionta*.

Arionta Traski, var. proles, Hemphill.

Shell umbilicated, very much depressed, thin, shining, of a dark horn-color; whorls 5½, somewhat flattened above, convex beneath, the last slightly falling in front, with a dark band above the periphery, and crowded with strong oblique striæ; suture well impressed; umbilicus moderately large and deep; aperture hardly oblique; peristome simple, thin, subreflected, its terminations approaching. Height ⅜ inch, breadth ⅞ inch.

Tulare Co., California, near Fraser's Mill.

A much flatter and more depressed form than any of the varieties of *Traski* that I have seen. There are no revolving microscopical lines, as in *Traski*.

The above is Mr. Hemphill's description.

Arionta tudiculata, var. Tularensis, Hemphill.

Shell umbilicated, very thin and frail, shining, of a light greenish horn-color, globosely depressed; whorls 5½, convex, the surface minutely granulated, and crowded with fine oblique striæ, with a single chestnut revolving band; suture well impressed; umbilicus very small; aperture oblique, subcircular; peristome simple, hardly thickened, its columellar portion expanding and nearly covering the small umbilicus. Height ⅝ inch, breadth ⅞ inch.

Tulare Co., California.

This is one of those puzzling intermediate forms uniting two species that can be with equal propriety placed in one or the other. It has the exact form of the typical *Traski* found at Los Angeles, and along the coast, though much smaller and thinner, and it has the sculpturing of *tudiculata* much modified. It seems to fill the gap quite completely between those two species.

The above is Mr. Hemphill's description.

Arionta tudiculata, Binney.

Plate II. Fig. 7, 8.

New figures are here given of the form *cypreophila*.

In The Nautilus, Vol. IV. p. 41, 1890, Mr. Hemphill also describes a var. *subdolus* thus: —

Shell narrowly umbilicated; globosely depressed, of a dark yellowish color, surface somewhat shining, covered with oblique striæ, interrupted by numerous wavy lines and oblong blister-like wrinkles, hardly perceptible to the naked eye; whorls 5½, convex, striped by a single chestnut band, double margined by lighter ones; spire very little elevated, suture well impressed; lip simple, reflected, and nearly covering the umbilicus, its terminations approaching and joined by a thin callus; umbilicus narrow and small. Height ⅝ inch, greatest diameter 1 inch, lesser ⅞ inch.

San Jacinto Valley, San Diego Co., California.

A very depressed form, quite variable in size, some of the specimens not being more than half the size of the measurements given. It is lighter colored than any of the southern varieties of *tudiculata* except var. *Binneyi*.

Arionta Ayresiana, Newcomb.

Plate I. Fig. 7.

I give a new figure of this species.

Arionta intercisa, W. G. Binney.

In "Zoe," Vol. I. No. 11, January, 1891, p. 330, Mr. Hemphill describes these varieties of *A. intercisa*: —

Var. *minor*. Smallest specimen, greatest diameter 18 mm., altitude 11 mm. Uniform light yellowish chestnut-color, with and without a band, and varies very much in form and elevation or depression of spire.

Var. *elegans*. Uniform ashy buff-color, faintly banded, and variable in form.

Var. *nepos*. Uniform ashy white; spire horn-color, variable in form and sculpturing.

Var. *albida*. Uniform milk-white, sometimes with a faint band at the periphery; sculpture nearly obsolete.

In the same journal (p. 434) Mr. Hemphill thus describes several varieties of *redimita*, which species he refers, however, to *Kelletti*: —

Var. *castaneus*. Uniform, polished, chestnut-color, darker band at the periphery, spire sprinkled with fine ashen specks.

Var. *hybrida*. Uniform ash-white color, and a dark band at the periphery, flecked with transverse markings and specks of dark brown and light chestnut.

Arionta ruficincta, Gabb.

Plate I. Fig. 3.

A new figure is given of this species.

Arionta Kelletti, Forbes.

Mr. Hemphill, in Terr. Moll. V., 3d Suppl., has thus described several varieties. I figure authentic specimens of each.

Var. *albida* (Plate IV. Fig. 3). This is a beautiful clear white translucent

vàriety, with no markings or stains of any kind. It is quite thin and frail, and a trifle smaller than the average size of *Kelletti*.

Santa Catalina Island, California. Two specimens only found by me.

Var. *castanea* (Plate IV. Fig. 4). Among the numerous patterns of coloring assumed by *H. Kelletti*, none are more conspicuous than this well marked variety. The body whorl is of a deep shiny chestnut-color above the periphery, and becomes lighter as it follows the whorls of the spire to the apex. The band at the periphery is quite variable in the different specimens; it is generally light and well defined above, but below it is irregular, and spreads over the base of the shell more or less.

Santa Catalina Island, California. This variety is not rare.

In "Zoe," Vol. I. No. 11, pp. 333, 334, Mr. Hemphill has also thus described several other forms.

Var. *nitida* (Plate IV. Fig. 2). Uniform, translucent, shining, dark horn-color, with a poorly defined dark band, coalescing with a poorly defined whitish band below it, at the periphery; spire faintly flecked with ashen gray.

Catalina Island.

Var. *multilineata* (Plate IV. Fig. 1). Shell marked by alternate shades of ashen white, chestnut, or brown, arranged in an irregular series of revolving and sometimes wavy lines, with a broader and poorly defined band at the periphery; markings finer beneath than above.

Var. *frater*. Shell of a beautiful, uniform, horn-buff color, sometimes fading into lighter horn-color, with a darker band at the periphery, and numerous faint, alternate revolving lines of ashen or dark horn-color above and below; generally, not always, lighter colored beneath, and sometimes with a whitish zone beneath the band at the periphery.

Var. *Californica*. The shell is colored with a darker shade of uniform buff than the above, dark band at the periphery, generally uniform in color above and below; sometimes flecked with squarish dots.

Var. *Forbesi*. Ground coloring whitish buff, with a revolving series of poorly defined and coalescing lines, bands, and blotches.

Var. *bicolor*. Color very dark horn or brownish, flecked with numerous revolving very fine dots or irregular lines, with or without a very faint band at the periphery.

Var. *tricolor*. Irregularly painted with numerous revolving whitish, brownish, and chestnut flecks, blotches, and stains, with or without a band at the periphery.

Var. *albida*. (See below.)

Var. *albida*, *a*. Milk white ground, very faintly stained with light horn, and with poorly defined and fading lines.

Mr. Hemphill considers *redimita* as a form of *Kelletti*. (See that species.)

Euparypha Tryoni, Newc.

Mr. Hemphill has thus described several varieties. (See Zoe, Vol. I. pp. 331, 332.)

Var. *varius.* The upper or dark zone is of a lighter shade of bluish brown or chestnut than the type, and is flecked and sprinkled with ashen white; band at the periphery dirty white beneath.

Var. *nebulosa* (Plate IV. Fig. 5). Lighter colored above than var. *varius*, marbled and clouded with various patterns of dark brown and dirty white; dirty white beneath.

Var. *fasciata* (Plate IV. Fig. 6). Uniform light chocolate above and beneath, with a dark band at the periphery.

Var. *Californica.* Creamy buff-color, darker above than below the periphery, very faintly banded.

Var. *albida.* Uniform creamy, and sometimes milk-white above and beneath, and without band.

Var. *subcarinata.* Among the subfossils that occur on Santa Barbara Island we find a form of *H. Tryoni* which adds an interesting link to its history and to its present form. It may be characterized as follows. Shell depressed globose, consisting of about $5\frac{1}{2}$ whorls, the last subcarinated at the periphery; in other respects closely resembling the recent form. Greater diameter 23.15 and 20.11 mm., largest and smallest specimens.

Pomatia Humboldtiana, Val.

Texas, at Altuda, at an elevation of 5,000 feet, where it, a single specimen in fair condition, had been thrown out with soil by a prairie dog. (Mus., No. 118,366.) William Lloyd.

This species has not before been reported from any locality within the territory of the United States. It was described from Mexico, where it is found in the neighborhood of the city of Mexico, and in other localities. The national collection contains several examples from the Real del Monte. It has a pretty close resemblance to some of the varieties of the European *H.* (*Pomatia*) *pomatia*, and it may possibly be an introduced form. *H. pomatia* has for centuries been esteemed as an article of food in various parts of Europe, and was regarded as a dainty by the ancient Romans. It was propagated and raised in large quantities for their use, and specially fed on certain plants to give the flesh a particular flavor.

Unmistakable specimens of another favorite edible snail common to Europe, *H.* (*Pomatia*) *aspersa*, is found in Mexico, and examples from Puebla, in the province of Puebla, Mexico, were presented to the National Museum by the Mexican Geographical Commission a few years ago. The presence of these two forms most certainly suggests the question as to whether they were not introduced by the Spaniards many years, centuries, ago, either for food purposes or incidentally in the routine and accidents of commercial intercourse.

The above was published by Stearns in Proc. U. S. National Museum, Vol. XIV. p. 96, 1891. It will be remembered that *Helix Buffoniana* was figured as *aspersa* by Dr. Binney in Volume III.

Bulimulus Ragsdalei, Pilsbry.

Plate II. Fig. 9.

It is about the size and form of *B. Mooreanus*, but rather more slender and elevated. The surface is not smooth, as in the other American *Bulimuli*, but strongly ribbed-striate longitudinally. The apex is blunt; peristome thickened within; columella reflexed over the narrow but open umbilicus. The aperture is less than half the length of the shell; color brownish, corneus somewhat translucent, the riblets opaque white. Height 22 mm., diam. 10 mm.; height of aperture 10½ mm., diameter 7 mm.

Bulimulus Ragsdalei, Pilsbry, The Nautilus, Vol. III. p. 122, March, 1890.
Proc. Acad. Nat. Sci. Phila., 1890, p. 296, Plate V. Fig. 3.

St. Jo, and at Warren's Bend, twenty-five miles from Gainesville, and in Cook and Montague Counties, Texas (Ragsdale).

A figure of an authentic specimen is given 1½ the natural size. The description is a copy of the original.

Bulimulis Dormani.

Plate I. Fig. 6.

A new figure is given.

Rhodea Californica.

This extralimital species has actually been received by Dr. Cooper from Lower California. (Proc. Cal. Acad. Nat. Sci., 1891, p. 102.) It had been quoted as an Achatina from Monterey. (See Vol. V.)

Pupa Californica.

Dr. Sterki in Nautilus, Vol. IV. page 7, mentions a variety, *elongata*, from San Clemente Island; on page 18, varieties *trinotata*, *Diegoensis*, and *cyclops*.

Pupa Coloradensis, Cockerell.

Shell brown, shiny, thinnish, striate, especially on penultimate whorl; outline oblong-oval, barrel-shaped; apex blunt; whorls 4; aperture pyriform; peristome brown, thick, continuous by a well marked callus on parietal wall; outer lip not constricted. The teeth within the aperture are brown, one long, one on parietal wall, one on columella, and two (the lower one largest) on outer wall. Long. 1½, lat. 1 mm. Allied to *P. corpulenta*, but decidedly smaller, more striate, and slightly narrower. (Cockerell.)

Pupa Pilsbryana, Sterki.

Shell minute, narrowly perforate, cylindrical-oblong to cylindrical, somewhat attenuated towards the rather blunt apex, colorless (when fresh glassy) with a very delicate bluish tint, smooth and polished, with few, irregular microscopic striæ which are more marked near the aperture. Whorls 4½–5½, moderately rounded with a rather deep suture, especially in the upper half, regularly and slowly increasing, the embryonal being relatively large, the last somewhat ascending toward the aperture; the latter of moderate size, lateral, subovate, margins approached, peristome somewhat expanded, without a thickened lip or a callus in the palatal wall; outside is a barely perceptible trace of a crest near the margin, and behind that a slight impression most marked upon the inferior palatal fold. Lamellæ 4 or 5; one apertural, rather high, of moderate length, simple; one columellar, horizontal, of moderate size, simple; basal very small or wanting; palatals the typical, inferior deeper seated, of moderate size, superior small or very small. Alt. 1.5–1.7, diam. 0.8–0.9 mm.

Pupa Pilsbryana, Sterki, The Nautilus, Vol. III. p. 123, March, 1890.

There is a slight variation; the example from New Mexico being of lesser diameter, and having no trace of a basal lamella.

The soft parts have not been seen so far, but will be of high interest, since, to judge from the shell, our species seems to be an intermediate form between the *hordeacella*, etc. group, and *P. curvidens*, especially its var. *gracilis*.

P. Pilsbryana has much resemblance in shape and size to small albino examples of *P. hordeacella*, Pilsb., but under a glass is at once distinguished by the shorter simple apertural lamella not ending at or very near the upper termination of the palatal margin, as it does in *hordeacella*, and by the smooth surface. The fine bluish hue may also be a distinguishing character if it prove constant.

The above is Sterki's original description.

Pupa calamitosa.

Plate II. Fig. 1.

See 3d Suppl., p. 219. A reduced copy of one of the original figures is given here.

Pupa Hemphilli, Sterki.

In examining a lot of about forty-five specimens of *Pupa calamitosa* from the banks of San Tomas River, Lower California, I found there were two distinct forms in them. The author says, in his description of *P. calamitosa:* "Several specimens have only one lamella on the outer lip, and are rather larger than the typical form described," represented in Plate XII. Fig. 16 (*loc. cit.*, No. 7). Probably I had a greater number of examples at disposition than Mr. Pilsbry. The two forms proved to be distinct by an entirely different formation of the lamellæ, as

well as of the basal part of the shell. And among the whole number I found not one intermediate or doubtful specimen. There is no doubt but that we have to consider them as being specifically distinct, the more so since they live together in the same locality. For the new species I would propose the name *P. Hemphilli*, in honor of the man to whom we owe so many valuable additions to our malacological fauna.

As in shape and general appearance the two species are almost alike, it may be the best way to characterize the one in question by comparing it with *P. calamitosa*, Pilsb. *P. Hemphilli* averages a trifle larger than its companion, but either is somewhat variable in size. While *calamitosa* has a minute perforation, *Hemphilli* is umbilicated in quite a peculiar way. There is a nodule-like projection on the umbilical part of the last whorl, producing a rima beside the umbilicus; in *calamitosa* there is nothing of this formation. On the other hand, the latter has a small but distinct groove-like impression just at the base, near the aperture appearing as a slight projection inside. This feature is wanting in *Hemphilli*. Lamellæ: in the latter species, when looking from front, only one is generally seen in the palatal wall, corresponding to the superior one in *calamitosa*, but longer; i. e. beginning deeper in the throat, and fairly seen on the outside; also marked there by a corresponding impression, ascending in a curve from near the base. A little distant from its inner end, just above the projection mentioned, there is another lamella beginning, directed toward the base and ending there, also seen on the outside. Quite generally there is a very small, thin, but well formed lamella in the palatal wall, near the projecting auricle. The columellar fold is quite short and small in *Hemphilli*, yet consisting of a vertical and a horizontal part. The (main) apertural lamella is decidedly longer in our species, and the supra-apertural higher and entire, while in *calamitosa* it is evidently composed of two parts marked by an indentation in the middle, or even entirely separated, in quite mature specimens.

About twenty examples, collected at San Diego, Cal., by Mr. Hemphill, are all *P. Hemphilli*, no *calamitosa* among them. They are little different from the San Tomas River specimens, except by a somewhat shorter palatal lamella.

The above is Sterki's description (The Nautilus, July, 1870, Vol. IV. p. 27). My figure was drawn by him from the type.

Pupa hordeacella, Pilsbry.

Plate II. Fig. 2.

The shell is of a long-ovoid shape, smaller and slenderer than *P. servilis*, Gould, translucent, waxen white, finely striate; the aperture is rounded, with a thin, expanded peristome. Within, there is, on the parietal wall, an entering fold arising near the termination of the outer lip, its edge a trifle sinuous or nearly straight; the columella has a fold about in the middle. There is a tiny deep-seated fold on

the base of aperture, near the columella, an entering fold within the outer lip, equidistant from the above described parietal and columellar folds, and a tiny denticle above it. The columellar fold is not situated so high on the pillar as in *P. servilis*. The latter half of the body whorl is flattened on the outer lower portion, as the Figure J shows. There is a low wave-like ridge or "crest" also, but scarcely visible in many specimens. Alt. 1.8, diam. 8 mm.

Pupa hordeacella, PILSBRY, Proc. Acad. N. Sci. Phila., 1890, p. 44, Plate I. Figs. G, H, I, J, K.

Arizona to Florida.

The figures were drawn with the aid of the camera lucida. They should be compared with Gould's excellent figures of *P. servilis* in the Boston Journal of Natural History, Vol. IV., Plate 16, Fig. 14, and those of *P. pellucida*, in Strebel's Beitrag zur Kenntniss der Fauna mexikanischer Land- und Süsswasser-Conchylien, Theil IV. Plate XV. Fig. 10. The latter are the more valuable in this connection, as they are not only faithful drawings on a sufficiently large scale, but are the only ones drawn from continental specimens (Vera Cruz, Mexico). The measurements given by Strebel and Pfeffer are, alt. 2½, diam. of last whorl fully 1 mm., alt. of aperture ⅔ mm. Gould's *P. servilis* and Pfeffer's *P. pellucida* were both described from Cuba. I see no reason for not following W. G. Binney in considering them synonymous, *pellucidus* having precedence. (Pilsbry.)

The above is Pilsbry's description. I give also a reduced view of one of his figures.

Pupa Clementina, STERKI.

Shell very minute, narrowly perforate, cylindrical, pale horn-colored, transparent, with rather obtuse apex; whorls 5½, regularly increasing, moderately rounded, with rather deep suture, smooth, with few microscopic striæ, somewhat shining; last whorl occupying rather more than two fifths of altitude, somewhat ascending to the aperture, with a slight, revolving impression on the middle of its last one third, ending at the auricle; a very slight, flat crest elevation near the margin, only in the lower part; aperture lateral, scarcely oblique, subovate with the palatal margin slightly flattened, upper part of same somewhat sinuous, peristome a little expanded with a slightly thickened lip just at the margin; lamellæ 6, white, two on the apertural wall, the apertural typical, and a rather long supra-apertural, ending in a callus at the upper termination of the palatal margin; columellar one typical, horizontal; basal very small, nodule-like, deep-seated; palatals two, typical, the inferior a little longer. Alt. 1.9, diam. 0.8 mm.; apert., alt. 6, diam. 0.5 mm.

Pupa Clementina.

Three examples of this species were collected by Mr. H. Hemphill on San Clemente Island, California, among numerous *P. Californica*, Row. All were exactly alike, well formed and fully mature. They cannot be referred to any one of our species published, and doubtless represent a form of their own, although so far it was not possible to examine the soft parts.

In size, shape, and general appearance it somewhat resembles *Isthmia*, yet lacks the rib-like striation; the lamellæ would be typical for *Vertigo* and some of the smaller *Pupæ* but for the presence of the well developed supra-apertural which *P. Clementina* has in common with *P. calamitosa*, Pilsbry, and *Hemphilli*, Sterki; but, on the other hand, there is nothing of the characteristic palatal or gular folds of these two species. Thus, in several regards, our form is an intermediate and connecting one between different groups, and consequently deserves our special interest.

Pupa Clementina, Sterki, The Nautilus, Vol. IV. No. 4, Plate I. Fig. 4, August, 1890.

The above is a copy of Sterki's original description and figure.

Pupa Dalliana, Sterki.

Shell conic or ovate-conic, of greenish horn-color, transparent, finely irregularly striate in the lines of growth, polished; whorls 4½, well rounded, with deep suture rather rapidly increasing, the last occupying about ⅔ of altitude towards the aperture, somewhat ascending on the penultimate. Aperture lateral, somewhat oblique, subovate, with just perceptibly flattened palatal margin; margins approximate, the ends protracted; peristome shortly but decidedly expanded, with a very fine thread-like lip near the margin, the same continuing as a very fine callus on the apertural wall inside of the line connecting the ends of the margins; palatal wall quite simple; no lamellæ. Alt. 1.2, diam. 1.3 mm.

Pupa Dalliana.

This form has been collected by Mr. Hemphill near Clear Lake, Lake Co., Cal., and I propose to name it in honor of Mr. William H. Dall. The specimens before me were fifteen, fresh, remarkably uniform in their whole appearance; all were more or less covered with a dark brown hard crust of slime and dirt, generally thickest around the aperture. Doubtless this coating is done "purposely" by the animals, as in many other species also. When cleaned, it shows about the size and shape of a well grown *Vertigo ovata*, Say; but by a good eye, or under a glass, is at once recognized as something else, by the rounded aperture and the absence of lamellæ. (Sterki.)

Pupa Dalliana, Sterki, The Nautilus, Vol. IV. No. 2, p. 19, June, 1890.

Dr. Sterki's description is copied above. My figure was drawn by him from the type.

Pupa syngenes, Pilsbry.

Shell subcylindrical but wider above, composed of eight narrow, convex whorls, *sinistrally convoluted;* texture as in *P. muscorum*, but color rather lighter brown. Last whorl ascending, imperforate, bearing a strong high crest just behind the

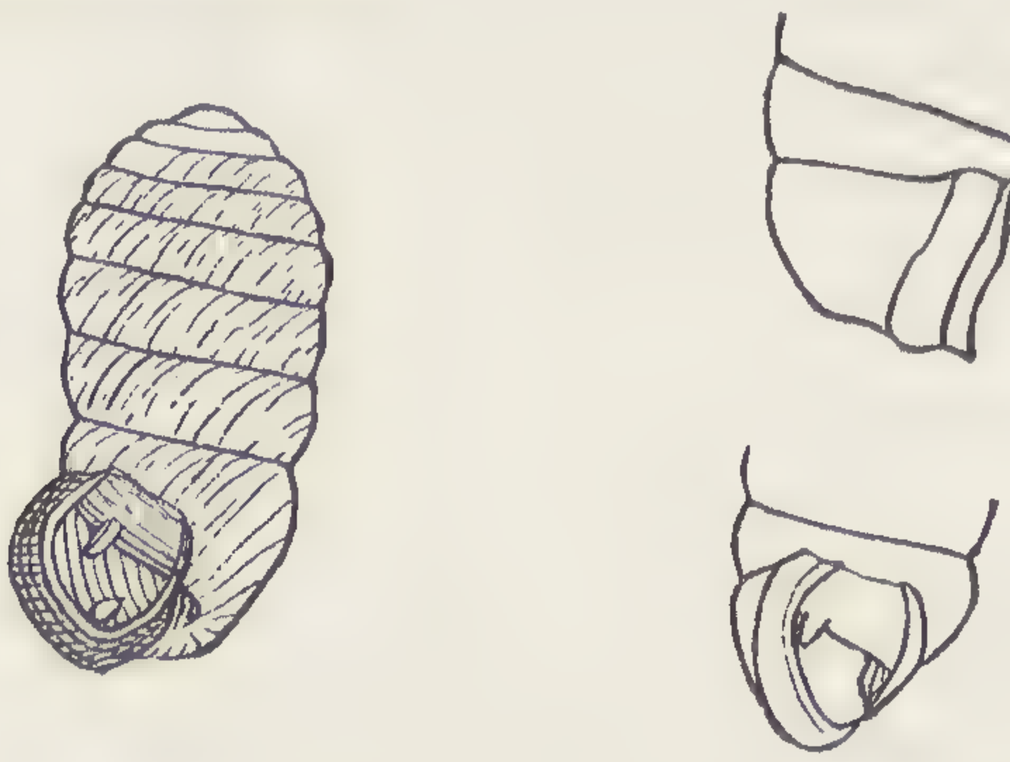
outer lip. Aperture shaped as in *muscorum*, having a single small parietal denticle. Altitude $3\frac{3}{4}$, diameter $1\frac{2}{3}$ mm.

Pupa syngenes, PILSBRY, The Nautilus, 1890, Vol. III. p. 296, Plate V. Figs. 1, 2.

Two specimens of this form are before me, and I am in doubt whether to give them a new name, as they may be only sinistral monstrosities of the common *P. muscorum*. The shells are labelled "Arizona" in the Academy collection, collector not known.

(Since the above paragraphs were in type, I have received a communication from my friend, Dr. V. Sterki, to whom I sent a specimen of *P. syngenes*, which I at first described as a variety of *muscorum*. He says: —

"I am satisfied that it is a species, and not a var. of *muscorum*; the shape of the whole shell, the last whorl so considerably flattened, and ascending, the number of whorls, seem to me to prove its specifical rank. . . . After washing out the aperture of your specimen, I saw a rather strong lamella or tooth on the columella, and a barely perceptible trace of an inter-palatal lamella, which, however, is validified by the impression on the outside.")



The above is Pilsbry's description. An authentic specimen drawn by Dr. Sterki is figured here.

Vertigo ovata, SAY.

Of *V. tridentata* Sterki writes (The Nautilus, 1890, p. 135): "It has a wide distribution in the northern part of the country; originally found in Illinois, it has been collected in different parts of Ohio and New York, as well as in Minnesota and Colorado. In general it is remarkably constant in its characters; yet there are slight differences; here I found a few examples from low ground, together with *V. ovata;* they were a trifle larger, with a thicker and deeper colored shell than those from upland places."

Vertigo Oscariana, Sterki.

This is the most peculiar of our species. It is of the size of *milium*, but oblong, with either end nearly equally pointed, the last whorl being considerably narrowed and flattened towards the subtriangular, small aperture; shell thin, delicate, of pale horn-color, as is the palatal wall and margin; the latter simple and straight, with a very slight, thin callus inside; lamellæ 3, whitish, rather small; one apertural, one columellar (longitudinal), and the inferior palatal; sometimes there is also a very small superior palatal. Length 1.5, diameter 0.8 mm.

V. Oscariana. $\frac{20}{1}$

This remarkable *Vertigo* has been detected in Eastern Florida, on the coast at Mosquito Island, etc., by Mr. Oscar B. Webster and his father, Mr. Geo. W. Webster, of Lake Helen, Florida. These gentlemen took much pains to ascertain the range of distribution of this form and some others, and it is consequently only just to name the species in honor of Mr. Webster. The most striking character of it, besides the narrowed last whorl, is the thin and straight palatal wall and margin, so that, indeed, the shell appears to be immature. But when seen under a glass of sufficient power, the margin is completed, and, as already mentioned, there is a thin callus at a little distance from the margin. Moreover, Mr. Webster wrote me that, of more than 150 examples he had seen, all were alike.

A few days ago, in a lot of *P. corticaria*, Say, from Ithaca, N. Y., sent from Texas, there was one example of this species, the shell dead, but in fair condition, a little larger and less fragile than the Florida examples, and with a well marked callus corresponding to a slight but distinct crest. The specimen may have been collected in New York, and from its appearance at least I would ascribe to it an origin north of Florida. Since the above was written, I have found a few examples in drift from Guadalupe River, Texas, collected by Mr. J. A. Singley, sent by Mr. Wm. A. Marsh.

By the kindess of Mr. Webster I was enabled to see a living example. The foot and the lower parts of the head are nearly colorless; head, eye-tentacles, and neck light gray. Jaw very tender, thin, pale yellow, consisting of about 14 longitudinal plates, shorter and wider in the middle, longer and narrower toward either end; it is much like that of *V. tridentata*, Wolf. Odontophore about 0.36 mm. long, 0.1 mm. wide, about 110 square rows in each $\frac{m}{3} + \frac{2}{3} + \frac{x}{4}$ teeth; central very small; laterals gradually passing into marginals; the latter serrate. Different from that of *V. tridentata*.

In drift with numerous minute shells, from Guadalupe River, Texas, kindly sent by Wm. A. Marsh, I found one specimen of this species, which consequently is not confined to Eastern Florida, where it was detected by Messrs. Webster, but may be widely spread over the southern part of our country.

Vertigo Oscariana, Sterki, Proc. Ac. Nat. Sci. Phila., 1890, p. 33; The Nautilus, 1890, p. 136.

The above is Sterki's description, and the figure is drawn by him from the type.

Vertigo Binneyana, Sterki.

V. Binneyana. $\frac{20}{1}$

They are of the size and general appearance of *V. callosa*, very narrowly perforate, cylindrical oblong, light chestnut-colored; whorls 5, moderately rounded, nearly smooth; aperture relatively small, peristome little expanded; outer wall with a well formed crest interrupted by a rather long revolving groove; corresponding to the crest there is a callus of lighter color; lamellæ 6; on the apertural wall a small supra-apertural and a well developed apertural; columellar appearing rather massive; at the base, one rather small but well formed, appearing tooth-like; palatals 2, long, especially the inferior. Length 2.0 mm., diameter 1.0 mm.

Last year, Mr. W. G. Binney kindly presented me with two examples of a *Vertigo* collected at Helena, Montana, by Mr. H. Hemphill, which seemed to be of a new species; but yet I did not like to publish a description founded upon only these two specimens. Lately among a number of small *Pupidæ* from different parts of British America sent by Mr. Geo. W. Taylor of Ottawa, there were a few examples of this same species, from Winnipeg, Manitoba, dead and weathered, but good enough to be identified.

Probably there are other examples of this species in collections, and more will be found in the Northwest. It is named in honor of Mr. W. G. Binney, to whom I owe the two beautiful specimens in my collection.

Vertigo Binneyana, Sterki, Proc. Ac. Nat. Sci. Phila., 1890, p. 33.

The above is Sterki's description. I am also indebted to him for the figure.

Vertigo callosa, Sterki.

There are in collections two different species under the name of *V. Gouldii*, Binn. Their size and coloration is nearly the same, at least in most variations, as are also the apertural lamellæ as to number and position. Yet they are decidedly and constantly distinct, especially by the formation of the outer wall at the aperture. Judging from the descriptions and more especially from the figures, the true *V. Gouldii* is the one characterized as follows: the last whorl is somewhat predominating, thus rendering the whole shell more ovate or conic ovate; the palatal wall near the aperture is decidedly flattened, or impressed, the impression comprising also the crest and being especially well marked at the "auricle" (as I name the more or less projecting part about the middle of the outer margin, to have a concise expression), forming a roundish groove outside and a decidedly prejecting angle inside, thus producing the "two curves meeting in the centre of the peristome." A feature not striking, but only seen by careful examination, is the position of the short tooth-like lamella at the base, somewhat nearer the margin than the end of the columella, the base perceptibly widened at that place; the said lamella is probably an equivalent of the inferior columellar lamella, which in most *Vertigos* stands very low, in many exactly at the base.

The other species, *V. callosa*, has the last whorl relatively less wide, so that the whole shell is of a more oblong shape. In the palatal wall, only the part behind

the crest is somewhat flattened, while the latter itself forms one unbroken curve from the base up to the suture, and at the moderately projecting auricle there is only a slight flattening. The inferior columellar lamella is at the end of the columella, sometimes wanting or a mere trace. Well worthy of notice is a pecuiiar formation of the surface, the epiconch showing microscopic wrinkles or foliations in the direction of the lines of growth producing a peculiar silky gloss, especially on quite fresh examples, and more in some forms than in others.

The first two examples of this species I obtained in 1885 from Mr. Henry Moores, of Columbus, Ohio, and in 1889 I saw a few more in his collection. In 1887, Mr. E. W. Roper sent me some others from Massachusetts. Last year in different collections I saw quite a number of specimens from different places in New York near the metropolis, under various names: *V. Gouldii*, *milium*, *ovata*, and also mixed with *Bollesiana*. Of the Ohio examples the color is somewhat lighter, the callus and the lamellæ are strong and white, while in the Eastern examples they are somewhat thinner and more of the color of the shell. The name *callosa* was thus mainly derived from the Ohio form (which, however, may be regarded as a variety).

It is with some hesitation, however, that I now bring it under this head; it is the equivalent of the European *V. pygmæa*, Drap., of which I have examples for comparison from different countries of the Old Continent, which I have partly collected myself there during a number of years. The two may even be identical; at least it would be absolutely impossible to distinguish New York examples from most Europeans. Both forms agree also in certain variations of the apertural lamellæ; the inferior columellar lamella may be absent in either, or there may be present a small supra-palatal fold, thus rendering the number variable from 4 to 6, the typical, however, being 5. An examination of the soft parts will probably decide the question; so far I have not had an opportunity to make it.

On our continent, the range of distribution of the two species — *V. Gouldii* and *callosa* — seems to be somewhat different, the former having been found in New York, Ohio, Illinois, and Colorado, the latter from Massachusetts to Ohio.

Vertigo callosa, Sterki, Proc. Ac. Nat. Sci. Phila., 1890, p. 31.

The above is Sterki's description.

Vertigo parvula, Sterki.

Among several hundred small *Pupidæ* collected in Northeastern Ohio (Summit and Lake Counties) by Mr. A. Pettingell, there were two examples of a doubtless new species, which I in the same way named *V. parvula*. It is about of the size, shape, and appearance of *V.* (*Angustula*) *milium*, Gould; but ranges in quite another group, having a quite simple palatal wall and margin, and only three lamellæ.

In Texas, *Vertigos* seem to be decidedly rare. In many hundreds of *Pupidæ* from that State which Mr. J. A. Singley and Mr. Wm. A. Marsh kindly forwarded me there were only about half a dozen such; a few *milium*, one *rugosula*, one *Oscariana*, as mentioned above, and one specimen of a form which probably will prove to be a new species of quite peculiar formation.

Vertigo parvula, Sterki, The Nautilus, 1890, p. 136.

The above is Sterki's description.

Vertigo approximans, Sterki.

In 1887, Mr. A. A. Hinkley, of Dubois, Ill., sent me, with other *Pupidæ*, one specimen of a *Vertigo*, probably new, and in 1889 another of the same. The said gentleman and Mr. William A. Marsh kindly forwarded me all their *Pupidæ* for examination, but so far I have found no other example, yet I am satisfied such will be found. The form is related to *Vertigo ovata* and *Gouldii*, but different, and is characterized by the two palatal lamellæ being close together, for which reason I gave it the manuscript name *V. approximans.*

Vertigo approximans, Sterki, The Nautilus, 1890, p. 136.

The above is Sterki's description.

Vertigo rugosula, Sterki.

Related to *V. ovata* and *Gouldii;* in shape more elongated than the latter, more cylindrical, and somewhat larger. Apertural parts and lamellæ much like those of *ovata;* but the columella is decidedly longer and straighter, and the inferior columellar lamella is distinctly placed on it. Length 1.8–2.0, diameter 1.1 mm. Of a peculiar formation is the surface. Of the five well-rounded whorls, about one and a half of the upper are nearly smooth; the following, with exception of the last, are distinctively and regularly striated; the last is very finely but distinctly rugose in the sense of the lines of growth, near the aperture again striated. Color, dark chestnut.

V. rugosula.

This is a beautiful species, of which I saw the first example in the collection of Mr. Bryant Walker, who had found it in April last at Pass Christian, Mississippi. Last September, Mr. W. G. Mazyck collected a number of them on Sullivan's Island, S. C. In either place they were in company of *Pupa rupicola,* Say. Quite lately I have seen one example from Lee County, Texas, sent by Mr. J. A. Singley; it was a dead shell, and not fully mature, but recognizable. The species consequently seems to be widely distributed along the South Atlantic and Gulf coasts. Two specimens were sent in by Mr. H. Hemphill, who collected them at Fish Camp, Fresno Co., Cal.

In Eastern Florida, Volusia County, etc., a form has been found to be quite common which I refer to this species, but as a distinct variety which may be called *ovulum.* It is somewhat smaller, ovate; the striation and rugosity of the surface are less marked, and the inferior apertural lamella is wanting. In turn it has in most examples a lamella at the base (between inferior columellar and inferior palatal), and the callus in the palatal wall is rather strong. The coloration of part of them is somewhat lighter. It cannot be confounded with *V. ovata,* Say, its relations to the type of *rugosula* being evident, and, in addition, *ovata* has been found with it. Nor can it be referred to *ventricosa.* It is larger and stronger, of much darker color, its surface is not so smooth and polished, it has three or even four lamellæ more, and the columella is longer.

Vertigo rugulosa, Sterki, Proc. Acad. N. Sci. Phila., 1890, p. 34.

The above is Sterki's description. The figure was drawn by him.

Liguus fasciatus, Mull.

Plate I. Fig. 5.

The Vaccas Key variety, noticed in page 435 of the Manual of American Land Shells, is figured in the plate.

Orthalicus undatus, Brug.

Plate II. Fig. 4.

I give a new figure of the variety of this species.

Holospira Arizonensis, Stearns.

Shell dextral, elongately cylindrical, pupiform, dingy white to pale horn-color, translucent. Number of whorls, twelve to thirteen. Slightly convex, the sutures distinctly defined. The upper six or seven whorls rather abruptly tapering towards the obtuse apex, which has a slightly twisted and rather a papillose aspect. The last whorl is curved under and constricted back of the mouth, forming an umbilical notch. The apex and following whorl are smooth; the three or four succeeding whorls sharply and somewhat obliquely plicated longitudinally, the median and following whorls becoming somewhat obscurely sculptured other than by distinct growth lines. The basal whorl is strongly sculptured below, and back of the mouth, and obtusely angulated underneath. Aperture ovate, slightly angulated anteriorly, somewhat effuse, rimmed and projecting. The dimensions of two examples are as follows: —

	mm.
Longitude	12½
Longitude	13
Greatest diameter	4
Greatest diameter	4

Dos Cabezas, Arizona, where the above two specimens and numerous fragments were found in a cave in November, 1889, by V. Bailey, and contributed to the United States National Museum (No. 104,392) by Dr. C. Hart Merriam.

Among the species of this group that are geographically related is *H. Remondi*, Gabb, described from Arivechi, Province of Sonora, Mexico, a form sharply sculptured throughout, and in minor features also different; *H. Pfeifferi*, Menke, collected by Remond at Hermosillo, in the same province, with the previouslv named

species; and *H.* (*Cœlocentrum*) *irregulare* of Gabb, from the high table-lands back of Mulege, in the peninsula of Lower California. All of these are separable at a glance from *Arizonensis*.

The above is Stearns's description and figure from Proc. U. S. National Mus., Vol. XIII. p. 208, Plate XV. Figs. 2, 3, 1890.

Onchidella borealis, Dall.

Coos Bay, Oregon.

It is gregarious in its habits. Fifty specimens were taken in a small crevice of clay shale, near high tide. Single individuals, or several clustering together, were taken afterwards lower down on the tide under loose stones. When in motion, the animal moves off quite rapidly for so small a creature, with two short, stout peduncles protruding in front of the mantle, bearing keen, sharp black eyes. The color is dark slate, splashed with blotches and streaks of ashen white. The body when in motion is $\frac{1}{2}$ inch long, $\frac{3}{16}$ wide, $\frac{1}{8}$ high, and oblong-oval in form, a little broader behind than before. It is covered with small tubercles, which are larger around the edge of the mantle than those higher up on the body, giving the edge of the mantle a serrated or tooth-like appearance when the animal is at rest. When it is at rest on a smooth surface, the base of the animal is nearly circular, or a little longer than wide, the centre of the body is elevated to quite a sharp apex, which together with its color resembles some varieties of a very young *Acmæa pelta*, and would be very readily taken for such by an inexperienced collector. The foot is white, and works in rapid undulations when the animal is in motion.

The above remarks are made by Mr. Hemphill in a recent letter.

EXPLANATION OF PLATES.

PLATE I.

Fig. 1. Anadenus Cockerelli. Animal and internal shell.
Fig. 2. Patula strigosa, var. Buttoni.
Fig. 3. Arionta ruficincta.
Fig. 4. Glandina decussata, var. Singleyana.
Fig. 5. Liguus fasciatus, var. from Key Vaccas.
Fig. 6. Bulimulus Dormani.
Fig. 7. Arionta Ayersiana.
Fig. 8. Zonites Simpsoni, enlarged.
Fig. 9. Binneya notabilis, enlarged.
Fig. 10. Same as Figure 2, toothed variety.

PLATE II.

Fig. 1. Pupa calamitosa, reduced from original figure.
Fig. 2. Pupa hordeacella, from original figure.
Fig. 3. Selenites Duranti, var. Catalinensis, enlarged.
Fig. 4. Orthalicus undatus, variety.
Fig. 5. Selenites Vancouverensis, var. Keepi, enlarged.
Fig. 6. Triodopsis Mullani, var. Blandi.
Figs. 7, 8. Arionta tudiculata, var. cypreophila.
Fig. 9. Bulimulus Ragsdalei, enlarged one half.

PLATE III.

Fig. 1. Limax Hemphilli, var. pictus. Animal and internal shell.
Fig. 2. Zonites Diegoensis, enlarged.
Fig. 3. Zonites macilentus, enlarged.
Fig. 4. Tebennophorus Hemphilli, jaw.
Fig. 5. Anadenus Cockerelli, jaw and tongue.
Fig. 6. Pristiloma Lansingi, enlarged.
Fig. 7. Zonites Caroliniensis, enlarged.
Fig. 8. Helicodiscus fimbriatus, var. salmonaceus, enlarged.
Fig. 9. Zonites sculptilis, enlarged.

PLATE IV.

Fig. 1. Arionta Kelletti, var. multilineata.
Fig. 2. Arionta Kelletti, var. nitida.
Fig. 3. Arionta Kelletti, var. albida.
Fig. 4. Arionta Kelletti, var. castanea.
Fig. 5. Euparypha Tryoni, var. nebulosa.
Fig. 6. Euparypha Tryoni, var. fasciata.
Fig. 7. Patula strigosa, var. bicolor.
Fig. 8. Patula strigosa, var. lactea.
Fig. 9. Patula strigosa, var. albofasciata.

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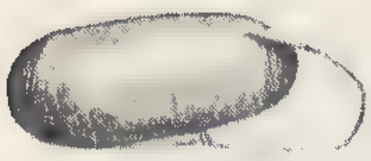

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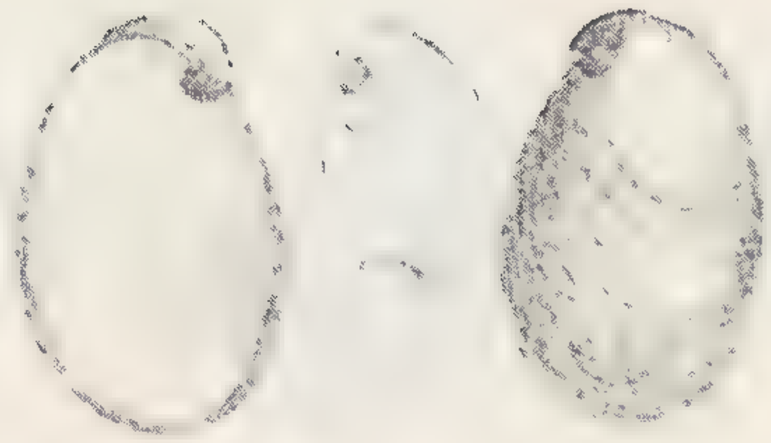

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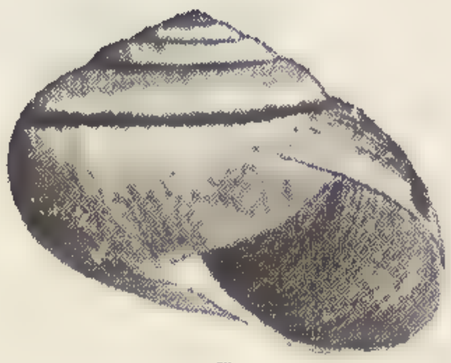

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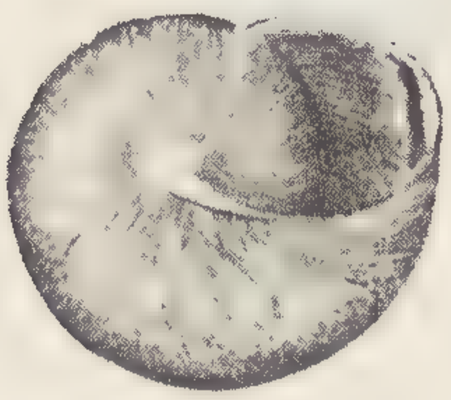

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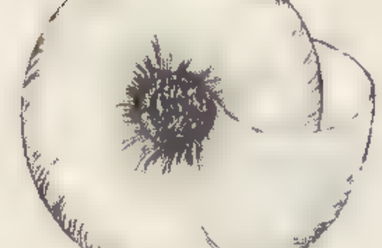

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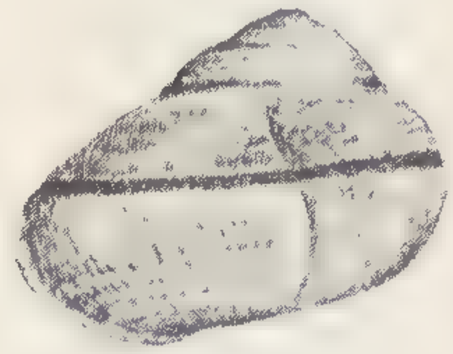

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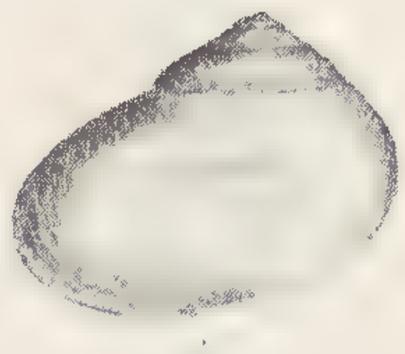

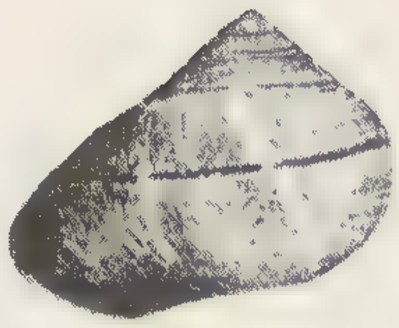

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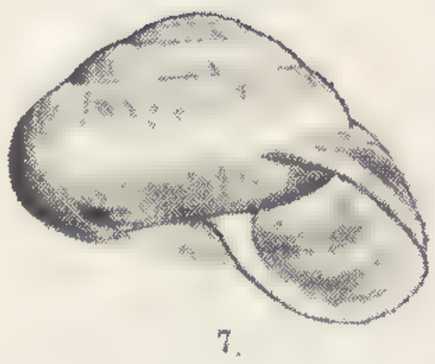

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